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WORKSHOP ON PLASTICIZERS

SCIENTIFIC ISSUES IN BLOOD COLLECTION, STORAGE AND TRANSFUSION
(Plasticizers in Blood Bags)

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MONDAY,

OCTOBER 18, 1999

The Workshop took place in the Masur Auditorium, National Institutes of Health, Bethesda, MD at 8:00 a.m., Jaroslav Vostal, Chair, presiding.

PRESENT:

JAROSLAV VOSTAL, M.D., Ph.D., Chair
TRACI HEATH MONDORO, Ph.D., Session Chair
RONALD BROWN, M.S., DABT, Session Chair
SUKZA HWANGBO, R.Ph. DABT, Session Chair
MELVIN STRATMEYER, Ph.D., Panel Chair
PAUL NESS, M.D., Speaker
JAMES AUBUCHON, M.D., Speaker
EDWARD SNYDER, M.D., Speaker
MICHAEL CUNNINGHAM, Ph.D., Speaker
ROBERT CHAPIN, Ph.D., Speaker
JOHN BUCHER, Ph.D., Speaker
VIRGINIA KARLE, M.D., Speaker
RAYMOND DAVID, Ph.D., Speaker
JOY ANDERSON, Ph.D., Speaker
RALEIGH CARMEN, Speaker

PRESENT (Cont'd):

JEFF MIRIPOL, Ph.D., Speaker
MICHAEL SHELBY, M.D., Ph.D., Speaker
DALAND JUBERG, Ph.D., Speaker
JOEL TICKNER, M.Sc., Speaker
NAOMI LUBAN, M.D., Panelist
KATHERINE SHEA, M.D., MPH, FAAP, Panelist
SCOTT PHILLIPS, M.D., Panelist
PETER ORRIS, M.D., MPH, Panelist
MAY JACOBSON, Ph.D., Panelist

A-G-E-N-D-A

A-G-E-N-D-A (Cont'd)

P-R-O-C-E-E-D-I-N-G-S

(8:19 a.m.)

CHAIRMAN VOSTAL: Good morning. I wonder if we could get started this morning. Hello, my name is Jaro Vostal, and I welcome you to the Workshop on Plasticizers, Scientific Issues and Blood Storage and Collection. We are running a little behind time this morning. We are waiting for Dr. Zoon. Hopefully, she will show up, and when she does show up, we will have her give her introductory speech at the first break.

I am glad you are all here to help us discuss these issues. They are two very important issues to FDA and CBER. There are a number of issues that concern DEHP; however, today, we are only going to concern ourselves with the issues that arise from blood collection and storage. And because we are short on time, I think we better get started. I would like to introduce Dr. Mondoro. She will be the moderator for the first session.

DR. MONDORO: Good morning. My name is Traci Heath Mondoro, and I will be chairing the first session, which is entitled Plastic Blood Bags. I have one announcement to make before we get the session started. If you would make sure that you pick up two supplement packets that are out on the table. These are

some more abstracts and biographies that can be put in your folders. There are, like I said, two packets that are paper-clipped, and they are out on the tables in the lobby.

The first session, the name pretty much says it all, Plastic Blood Bags. Our first speaker is Dr. Paul Ness. He is the Director of Transfusion Medicine Division at Johns Hopkins, and he is going to give a historical perspective and overview. As he is coming up, I would also like to remind you that today's meeting is being transcribed. So that if you do come to the microphone to ask questions, we ask that you state your name and your affiliation, so that it will be part of our public record. Thank you.

DR. NESS: Good morning. It is nice to be here although I had a lot of second thoughts after I agreed to give this talk. I guess I have reached the point in my career where I am asked to do a historical introduction rather than trying to present anything I really did myself. But as you will see as I give my remarks, this has been something that I have been interested and involved in for quite some time. So I am actually very happy to be here.

When I started trying to do the idea of doing a historical introduction about DEHP and blood

bags, I looked back into some of the medical literature for sort of reviews of this topic. Because if you look in the current blood bag text, you won't find very much in terms of the issue of phthalates in blood bags. People seem to think that the problem has gone away and it no longer really needs to be discussed.

So I picked up this book. It is a book called The Red Blood Cell, which was edited by Dr. Douglas Surgenor, and I will read to you a section from it briefly in what was called "The Historical Introduction." It says, "It is necessary to incorporate a plasticizer with polyvinylchloride polymer to provide the flexibility, toughness, ease of sealing and manipulative qualities needed in a blood bag. The added plasticizers have been in the phthalate group with DEHP a common choice. Adverse findings which demonstrate that significant quantities of phthalates leach out from the material of the bag have directed a search for other materials for bag fabrication. There have been many alarming reports that phthalates can migrate from polyvinylchloride blood bags into stored blood and localize in human tissues. The ability of man to metabolize phthalates remains unclear, and the overall biologic impact of the phthalate plasticizer is still unresolved. Acute effects of phthalates have not been

clearly demonstrated, but potential teratogenic and other long-range toxic effects are of great concern."

This was published in the early 1970's, and I thought it was a very well written statement now and actually at the time, because I actually wrote it. This is the first thing I ever wrote as a person who came to this campus and worked in what is now the National Heart, Lung and Blood Program. And I think you will see that we haven't actually moved that far beyond that unfortunately.

So in reviewing sort of the real early history, I think most of us in this audience are aware -- there may be a few people who don't know that much about blood bags, but just to cover them. The early history is that vinyl plastic bags were introduced sometime around 1950. Walter is given credit for that. It was shown that the survival of red cells stored in these bags was actually improved compared to glass bottles. And we all have seen that there are major advantages in collection, processing, storage and dispensing of blood components, particularly in platelet concentrates as a result.

An old friend here for some of us -- I guess I was in the field long before we were using this, but some of you out there may remember these more fondly, and obviously we have now moved to this type of arrangement

with plastic bags, different plastics to facilitate, for instance, platelet storage as opposed to red cell storage, and it really has allowed us to make a number of different blood components from whole blood. It has allowed us to facilitate aphoresis collection for various blood components, stem cell collections and a whole host of other kinds of medical things that have made transfusion medicine a very growing discipline.

Again, to review, unfortunately though a plasticizer needs to be incorporated. So that for the blood to be pliable, vinyl plastic containers require the addition of a plasticizer at levels of up to 20 to 30 percent of the final weight. And DEHP, di(2-ethylhexyl)phthalate, is a common choice for most of the medical plastics. DEHP is not chemically bound, but is dissolved physically in the plastic film. Initial studies when these bags came out implied that there were trace amounts of these materials which went into the bags when they were filled with anticoagulants. These initial results seem to be reassuring, but later other results came out which were a little bit more alarming.

This is a slide that actually I was able to borrow from Bob Rubin, which shows some of the original work that he and a graduate student, Rudy Jaeger, at Johns Hopkins did a number of years ago. He was looking,

actually, at an isolated chamber to isolate livers and do profusions of the livers and using a chromatographic technique when he found what he called in one of the profusion studies an unidentified compound compared to these other peaks that had easily been identified. To hear Bob tell the story, which is always a very entertaining event, this unidentified compound had been obtained from a profused rat or mouse liver, so the amount of blood in which to do biochemistry on this was very, very small. And biochemistry then is not what biochemistry is now. In any event, he decided that it would probably be a good idea to try to scale up this apparatus so that they could get enough of this material to actually analyze and find out what it was. So they went into a more macro system and actually came over to the Hopkins blood bank, because he said, well, we have a lot of outdated blood there and we could use the outdated blood to profuse the system. According to Bob, these are actually some of the first bags that they borrowed or took from the Hopkins blood bank as outdated blood to study. And when they did these experiments in a larger system, Rudy Jaeger apparently came to Bob and said, well, I have good news and bad news. In the larger system, I certainly can find the compound which you were interested in, that compound X. Unfortunately, it is

also there in heavy quantities in the starting material.

This then obviously became, after about a year of biochemistry, identified as DEHP, and Jaeger and Rubin reported initially in Lancet and later on in The New England Journal in the 1970's about contamination of stored blood with DEHP at levels of 50 to 70 milligrams per deciliter. It was also shown in this later article that it migrated substantially during storage, so that the migration rate they calculated was 2.5 mg/liter of blood for 24 hours of storage, such that one could get a possible dose in a bag of blood of almost 300 mg or about 5 mg per kilogram for an adult and even higher dose for a child, and these doses, as you will hear later, had been attributed or suggested that they may have some toxicity in some of the animal models.

Bob went on to work with Charlie Schiffer doing some actual measurements in platelet transfusion recipients, and they reported in Transfusion in 1976 that when platelet transfusion recipients were getting platelets, they actually had an intravenous injection of 26 to 62 mg of DEHP in the platelet recipients.

This, for those of you who haven't met him, is Bob Rubin at a younger day. He is actually in the audience today, and I am sure that we will be blessed by some of his comments as the day goes on. His observations

that I have talked to you have since obviously been confirmed by many laboratories around the country. What added, unfortunately, to some confusion about what was the role of DEHP in blood bags, however, were reports of widespread environmental contamination with DEHP. So how to place this transfusion problem into perspective became a difficult endeavor.

One of the things that happened in the 1970's and how I sort of got involved a little bit was that a number of studies were actually funded by what was called the National Blood Resource Program on this campus, and now it is part of -- it was part of what was then the Heart Institute, then the Heart and Lung Institute, and now the Heart, Lung and Blood Institute. But these studies were actually funded by NHLBI, which had some industrial studies, some studies by the military and studies by the private sector. Many of these have been reviewed in an international forum which was published in Vox Sanguinis in 1978.

Obviously I don't have time to go through that whole review, but you can see that there were various flavors of sort of reports that came out at that time. The industry studies showed that when they looked at tissue residues in transfused recipients, those studies were essentially negative. They showed that

platelet storage did not appear to be effected. They didn't show any increased particulates in bags stored with DEHP, and they emphasized the importance of making the DEHP a solution rather than an emulsion, which sort of clouded some of the studies about the vehicle in which DEHP was administered to laboratory animals.

The military published some excretion and metabolism studies and gave the implication that since these seem to be relatively rapidly metabolized, they would not be likely to cause a problem for most human recipients. On the other hand, there was a very intriguing report by Dr. Sherwin Kevy from Boston Children's Hospital, where he used a monkey experiment and these monkeys were given chronic platelet transfusions on a schedule which was not very different from what human recipients could be given, and showed direct evidence of hepatotoxicity in the monkeys who were being transfused with platelets which had been stored in the DEHP-containing container.

So there were studies that implied not much problem, maybe a problem, and it wasn't exactly clear where to go from here.

Well, at this point in my career, I came on to Johns Hopkins and actually had the opportunity to meet and actually work with Bob Rubin directly. This was

something that turned out to be a lot of fun and very intriguing in terms of this issue. When I first got there, Bob and I worked with a graduate student, who was going for a Ph.D. thesis, and he had some preliminary -- they had some evidence that when rats were given DEHP-containing infusions, they developed a DIC-type picture with fibrinogen activation and the generation of fibrin-split products.

So we decided to do some studies that compared blood which was stored in blood bags versus blood which was stored in glass bottles at the time. We had some very interesting results. When we looked at whole blood, in the bottles there were no evidence of any fibrin-split product generation or no fibrinogen activation. Whereas in the plastic bags, we did have fibrin-split products by clinical assay and evidence of fibrinogen activation. We also tried to make platelets and plasma and store them in glass bottles or plastic bags. Platelets obviously stored in a glass bottle were difficult. But we again found that fibrin-split products were found in blood stored in the plastic bags, and there were higher titers that were actually found in the platelets than in the native plasma, implying that cells in the medium had some additive effect in terms of the leaching or fibrinogen degradation.

We concluded in an abstract that we published at that time that blood stored in plastic bags in current use is not maintained in its native state. We actually presented these results at the AABB. We presented similar results at the American Heart Association in a toxicology meeting, but were never able to get them published in a peer review journal. These results sort of intrigued us and made us concerned that perhaps -- or at least me concerned that perhaps recipients of massive transfusions, where they have already been known to have a DIC-like picture sometimes, or people who had massive transfusions and had pulmonary failure, the so-called ARDS syndrome after a transfusion, that perhaps the DEHP storage media was having some sort of effect.

And I worried about this a little bit, but not too many other people worried about it too much. Everybody, at this point, started worrying about something else, which was the HIV epidemic, and I think that the sort of plasticizer issue sort of went away for a number of years. It actually went away, at least for me, for a number of years until the late 1980's, when Bob called again and said that Jeff McCullough, the editor of Transfusion had asked him to write a review on the status of blood bags. And that since he hadn't been that much

involved in the use of blood bags for a number of years, would I be willing to write or help him write this article.

So we wrote an article which was published in Transfusion called "What Price Progress", and what we showed is results that I am sure many of you are already aware of. We showed or reported that there was a low, acute toxicity for DEHP. But we did say that there were pulmonary reactions in animal models that were somewhat troubling. We quoted a number of papers from around the world showing suggestive evidence of chronic effects, including infertility, teratogenicity, carcinogenicity, hepatotoxicity, and cardiotoxicity. We, on the other hand, acknowledged that even though these effects might be deleterious, it was clear that DEHP had since been shown to have some benefits, actually, for red cell storage. It seemed to enhance red cell storage, which I am sure Dr. AuBuchon will talk about in the next talk. And in the conversation or in the article, we talked about further discussion and perhaps new solutions that might be available.

Soon thereafter, a plasticizer or a plastic bag system was released by the Baxter company called PL2209, which was a plastic storage system without DEHP introduced in the early 1990's. Now I am sure the

immediate assumption of anybody who read our paper was that we were being paid in some way by Baxter and were aware of this development and we were just writing this at that time to promote this release of this new blood bag system. And I can tell you that nothing was further from the truth and that when we wrote this paper, we didn't know anything at all that industry was actually working on a blood bag substitute that did not have DEHP.

I think it is fair to say, though, that even with our article, which we thought expressed appropriate concerns, there seemed to be little enthusiasm. I think I have used the term sort of collective inertia generated by transfusion services, perhaps because clear-cut human toxicity had not been identified and widespread acceptance was, at that time, inhibited by higher costs. These systems were introduced into a number of blood bags but have since been actually withdrawn from some of them or many of them because of the higher cost of implementation.

Well, I think it would serve as a good summary for this sort of historical introduction to sort of read the final paragraph of what we said in our "What Price Progress", because I think it is actually kind of an interesting summary, particularly for this meeting

today. What we said was on the basis of the available data, we believe that DEHP problems should be addressed in the following ways. Because much of the data suggesting toxic effects of plasticizers remain unknown to physicians and their patients, we would suggest that these data and the resulting issues be presented and discussed at a forum such as an NIH consensus development conference. We would anticipate that this type of public exposure would result in a call for more research in this area with emphasis upon the clinical study of multiply transfused recipients to determine if any evidence of toxicity can be found in humans. Another focus of this type of meeting would be the consideration of the status of blood collection systems without DEHP. The practical and regulatory issues that would confront any new blood bag system could be addressed, and the likelihood of substitute systems becoming available in the near future could be presented.

While we proposed this meeting actually in 1989, it is now 1999, and I guess we are just 10 years too late. But hopefully it is never too late, and I personally am very pleased that we are now having a meeting to sort of discuss these issues and come to grips with what the appropriate cause or causes and courses ought to be. Thank you very much.

DR. MONDORO: Thank you, Dr. Ness, for that overview and introduction. Now we are going to get a little bit more specific. Our next speaker is Dr. James AuBuchon. He is the Medical Director of the Blood Bank and Transfusion Service and professor of pathology and medicine at Dartmouth-Hitchcock Medical Center.

DR. AUBUCHON: Good morning. If I could have the first slide, please? I too appreciate the opportunity to speak before you today. It was fun to go through some old data and some old reports, which frankly many of which I had forgotten about, to return again to the issue of what does this plasticizer do with red cells.

Depending on your point of view, this is either the villain of the story or the hero. Its characteristics certainly have not changed in the last two or three decades. We know that this plasticizer is not covalently bound within the polyvinylchloride plastic, and it can indeed leach out. And this information, as Paul reviewed, has been well known in the literature for a number of years. This is not -- this compound, obviously, is primarily lipophilic and does not dissolve very well in crystalloid solutions. But if you put protein or lipoproteins or perfectly plasma in contact with polyvinylchloride containing DEHP, this

compound will very rapidly appear in the blood or the profusate.

The amount that accumulates over storage varies depending on how you assay it and exactly how the blood is stored and what blood component is being stored, but certainly a measurable amount does occur in blood during normal blood bank storage. The majority of DEHP appears in plasma, probably in association with albumin or lipoprotein, but somewhere between 5 and 10 percent does end up being associated with the red cells. And this red cell take-up of DEHP occurs quite quickly. Gail Rock was able to show that within minutes, a large proportion of the available DEHP could be found attached to red cells and approximately equal proportions of that DEHP were found in the red cell membrane and the red cell cytosol.

Of course, when the DEHP is transfused, it can be measured, as was just mentioned, and we will probably be hearing more about that today -- exactly what happens to DEHP and what it causes on its way to metabolism and disappearance.

The studies that were mentioned from Boston Children's indeed attracted a lot of attention in the blood banking world because of the potential for chronic exposure to DEHP having some detrimental affect to our

patients. This was in an era where we were not used to having a lot of public scrutiny as to what we were doing in blood banking, and frankly this escaped public scrutiny as well. It wasn't until after the era of AIDS that blood bankers became very accustomed to having the public pay attention to everything that we did. But blood bankers at this point still were concerned that the chronic exposure to DEHP may have a negative effect.

But on the other side of the coin, there was clear recognition that DEHP may be doing something good, and I will be spending the next few slides going through some of the data that were available back at that time, in the 1970's and early 1980's, detailing exactly what DEHP was doing for red cells. In fact, the more recent report of the Blue Ribbon Panel concluded that DEHP imparted a variety of important physical characteristics that are critical to blood storage, and that is indeed true.

As we mentioned from the early times of plastic blood storage, it was understood that these plastic bags were at least as good as glass bottles, if not in some ways better than glass bottles for long-term storage of red blood cells. The initial studies with plastic bags when you look at them today did not necessarily meet the same scientific criteria. There

were not good control groups, and to my eye anyway, it appears that the plastic bags of the mid to early 1950's were probably a little bit better than glass bottles in storing red cells, but it is difficult to say that with a P value in any true scientific confidence.

However, there are some data that we can indeed hang our hat on and that suggest that PBC with DEHP was better than glass containers. For example, after storing whole blood for 21 days in ACD and then determining at what saline concentration the red cells would completely hemolyze, it appeared that the PDC container stored red cells were more resistant to osmotic lysis than those stored in a glass container. Similarly, the plasma hemoglobin levels were found to be lower in those units of blood that were stored in the presence of DEHP than those stored in the glass containers. These are not proof absolute that the red cells are going to do better after transfusion, but they certainly are suggestive.

These initial concerns about the toxicity of DEHP and initial indications that DEHP may be doing something good for red cells prompted a number of in vitro studies. I will review a few slides here from Tim Eslep's work from Baxter. Baxter was obviously very interested in detailing exactly what DEHP was doing. And

in the studies that his group performed, they took CPDA-1 red cells and stored them -- either stored them not in contact with polyvinylchloride, either with the buffer with an emulsifier or with an emulsifier that had emulsified within it DEHP. And they looked at a number of in vitro parameters in an attempt to determine what the plasticizer may actually be doing.

They noted that when the red cells were stored in the presence of DEHP but not in the presence of the buffer or just the emulsifier, that the morphology was better maintained throughout 35 days of storage, and that the plasma hemoglobin level did not rise nearly as rapidly as when DEHP was not present. Again, this was not due to emulsifier. It was due to the DEHP, it appeared. And indeed when they looked at a number of other compounds, including metabolites of DEHP and including MEHP and ethylhexanol, they were able to show that these metabolites singularly or in combination did not produce the same effect on morphology or hemolysis that the DEHP did. So it appears that the DEHP was, indeed, in some ways assisting the red cells surviving the storage period.

Interestingly, if red cells were first stored without the presence of DEHP and then DEHP was added in a solubilized form part way through the storage,

the changes that were otherwise occurring were reversed. So here we see, for example, the effect of DEHP on morphology. This is the red cell morphology when DEHP is present, better maintained than when DEHP is absent. But when DEHP was added after two weeks of storage without DEHP, the morphology very quickly becomes that of the red cells that had been stored always in the presence of DEHP. That suggested that there was something physical that the DEHP was doing inside the red cell, which was not necessarily a metabolic-driven event. And indeed all of the standard metabolic indices that one looks at during red cell storage were just as well preserved with emulsifier as with DEHP. However, there was a difference in the amount of microvesicle formation during red cell storage when DEHP was added. So it appeared that in the presence of the plasticizer, there was less budding off of the membrane and less loss of membrane during storage, and that that may indeed be responsible or in some way related to the preservation of morphology and the lower hemolysis in the presence of this plasticizer.

Now the only recent study that I was able to find on this issue was published earlier this year from India looking at manufacturers' plastic bags that included DEHP compared to glass bottle storage. This study appeared to indicate that the ratio or the amount

of cholesterol and phospholipids during the storage was better maintained and was more normal, I guess you would say, in the presence of DEHP than in its absence. So although the cholesterol concentration appeared to increase and the phospholipid concentration appeared to increase during storage, that increase was not as great in the presence of DEHP as in the storage without DEHP.

A number of other groups were involved as well from the New York Blood Center. Some essentially dose response studies. Whether you looked at plasma hemoglobin or osmotic fragility, that the change that was seen over storage was less in the presence of DEHP. And as you increase the amount of DEHP, there appeared to be more beneficial effect there. So the more you put into these red cells, the more plasticized they became, if you would, the happier they appeared to be during storage. Indeed, here is a dose response curve done in parts per million showing that the greater the concentration of DEHP to which the red cells were exposed, the lower the hemolysis during storage.

This prompted us to conduct an in vivo study. These were all interesting in vitro phenomenon, but did they have any bearing to what was going on in the patient. This study, actually conducted back when I was a fellow here, was interesting to review again. We took

whole blood from normal subjects and stored it in PVC plasticized with TEHTM, a so-called non-leachable plasticizer paired with the same individuals storing their whole blood in DEHP plasticized plastic. In another arm of the study, these same subjects just stored their whole blood in glass or glass to which DEHP was added. These glass containers to which DEHP were added were glass bottles. We had to manufacture the CPDA-1 outside of any plastic containers to make sure that we did not have any DEHP contaminating the system through the anticoagulant. And then weekly, DEHP was mixed with an aliquot of autologous plasma that had previously been stored frozen. The DEHP was solubilized in the plasma. A measured amount of that plasma, in order to deliver the appropriate amount of plasticizer, was added on a weekly basis to those glass bottles to mimic the accumulation during storage of DEHP.

Another study performed later looked at red cells, where again in a paired fashion subjects stored their red cells in either non-DEHP plasticized plastic or with DEHP. In all of these three sets of studies, the 24-hour recovery of radio-labeled red cells at the end of a 35-day storage period was better in the presence of DEHP than when it was not included in the formulation. Some of these differences are indeed clinically

significantly potentially as well as all being statistically significant.

The difference in the curves appeared in the first few minutes. If you look at the T50 of the disappearance of the radio-labeled red cell, there is a marked difference in the cures just in the first 10 minutes. The difference appeared to decline after that. So the primary difference was immediate clearance of the red cells, which was greater without the presence of plasticizers in the bag.

This is shown here that from about the -- after the first few minutes clearly there was a flattening out of these curves. And between the 60-minute and 24-hour points, the curves were almost parallel. So the difference might be attributed -- I say might, we didn't actually look at this -- to increase rigidity or some other physical factor which led to earlier removal of the non-plasticized stored, non-DEHP-stored red cells.

If you then calculate this out to 24 hours, assuming approximately the same long-term survival -- which is standard in blood banking to assume that if red cells survive the initial time period, they will probably have a normal life span, you would predict that there is a 17 percent difference in red cell availability, which

is really attributable to this difference in what is occurring very rapidly after transfusion.

What exactly is going on here? This answer has never been defined Maybe that there is better preservation of phospholipid asymmetry, which is important in preventing microvesicle formation or which is associated with reduced microvesicle formation . It may be the plasticizers in some way interacting with the red cell cytoskeleton to counteract any effects of oxidation or detachment of the cytoskeleton, which would also lead to increased microvesicle formation. It may be that there is less availability of divalent cations, particularly calcium, to interact with the red cell member -- again, to cause effusion of these little microvesicle buds which can form.

So exactly what is going on here, we are not certain, but it does appear that there is some relationship between the presence of DEHP and the membrane directly.

Well, if DEHP confers some benefits to red cell storage but there are some risks associated with its use, is there something else that we can do? Could we use less of it? Is there some other plasticizer that could be used? A reduction in dose would appear ultimately to be problematic. Not only would the bags

become stiffer and potential for breakage increase during component production, but the dose response curves from the New York Blood Center studies would suggest that we could get to a point where we would not see the benefits that we had become accustomed to.

What about switching to a system in which there is less plasma? That is indeed what happened about this same time, where we switched from using whole blood primarily or packed cells to additive systems. And indeed, when you go from a whole blood system to an additive system, there is less accumulation of DEHP during storage. That may well be because there is just less plasma there and much of the DEHP is solubilized in the plasma. However, interestingly, although the total amount of DEHP is lower in an additive system unit, there is more actually in the red cells. It may be that there is less competition from proteins in the supernatant and the fluid surrounding the red cells and more of the DEHP is able to get to the red cell, where it is actually providing some benefit. We are not aware of any benefit of the DEHP being dissolved in the plasma. This is entirely conjecture. We don't know this for a fact. But it is interesting that we were able to accomplish a switch to an additive system which does provide better storage of red cells and longer storage of red cells than

a whole blood or a packed cell system, and possibly this is part of the reason that it does so.

Now as Paul mentioned in the previous talk, we do have other plasticizers available, and the butyryl-n-trihexyl citrate plasticizer, BTHC, which is part of 2209, has been available in the United States. Certainly, you can get 35 or 42-day storage of red cells with the appropriate anticoagulant. There appears to be no demonstrable difference between the 2209 and 146 plastic bag storage of red cells. How can that be if this is not a plasticizer that is doing the same things as DEHP. It clearly is not seemingly doing anything inside the red cell. These metabolic parameters are the same as one would expect with DEHP. The hemolysis is the same as one would expect with DEHP, and the recovery is about the same. Is there something else going on? That has never been finely determined. But it does appear that there is at least one plasticizer which does the same thing or provides the same environment for red cell storage that DEHP does.

We have some problems with this plasticizer, as was mentioned. It does have -- the bags do have what some regard to be an objectional odor. There is an increased cost and it does have increased oxygen permeability, which is not necessarily a down side for

red cells, per se, but it is just a different characteristic, and blood bankers did have to get used to having blood bags that were bright red as opposed to darker red with the use of 2209.

The question of which plastic to use is one which I think others will be talking about later. But PDC, plasticized with DEHP, is one that we have come to know and learn how to use very well in blood banking because of a number of very positive characteristics. These same characteristics are not present in other plastics that are available to us. So it does appear that the polyvinyl chloride family is one that we have been able to use successfully over the last three decades. The question of which plasticizer should be in that polyvinylchloride is another issue. Clearly, the DEHP which has been there for the last several decades is providing a benefit for red cells, and we cannot immediately remove DEHP and replace just any other plasticizer or use a non-leachable plasticizer. Because the red cell storage characteristic will indeed change, and we will not be able to store red cells for as long as we have in the past or with as good an outcome after transfusion.

So we clearly have benefits associated with DEHP. We have risks that are toxicologic in nature. The

Alternatives are not perfect and I look forward to today's discussions to determine where we should go next. Thank you very much.

DR. MONDORO: Thank you, Dr. Aubuchon. Our next talk is the final talk in this session on plastic blood bags, and it will be given by Dr. Edward Snyder. Dr. Snyder is a professor of laboratory medicine at Yale University Medical School and Director of the Blood Bank aphoresis service at Yale New Haven Hospital. I would also ask at the end of Dr. Snyder's talk if all three speakers could be seated at the panel table so that we can have a short question and answer period after that.

DR. SNYDER: I'm talking about platelets. This -- my talk is about plasticizers and platelets. For several years, there have been a variety of alternative plastics available for platelet storage. What I am going to do is to go through in my usual rapid flicker-fusion type of approach to try to cover as much data as I can, the purpose of which is to show the industry and the public that the blood bank community has available several different types of plastics and plasticizers which are shown to appropriately store platelets, and I wanted to provide some of that data and put some of this in perspective.

This is a picture of a platelet. What we

are concerned about is not only all the biochemistry inside the platelet, but what effects there are on the membrane. There does not appear to be same effect on platelet membranes as there is on red cell membranes. That is, it has not been shown that DEHP has a beneficial effect on platelets and platelet survival.

This is just electron micrograph showing similar kinds of things. We are concerned about not only attachment to receptors in the membrane, but also the release reaction whereby the various hemostatically active materials in the alpha granules and also the dense bodies, ADP and serotonin, can get to the outside by merging with the surface collecting system, which although it looks like a vacuole actually is an evagination of the membrane. The whole purpose of platelet storage is to collect the platelet, store it in a plastic bag, and then have it function during transfusion as well as it would if it were a fresh platelet.

Platelet storage bag suitability has a variety of characteristics that have to be evaluated. It has to have acceptable O_2 and CO_2 gas exchange, which is critical. The pH should be above 6.0 at the end of the storage period. Right now in the CFR, it is 6.0. But in new guidance that has been let out to the industry for

comment, the suggestion was made by the FDA that this should be raised to 6.2, which people working in the field applaud, because 6.0 is too low.

In vitro characteristics need to be measured. Radio-labeled in-vivo characteristics are also evaluable. In-vivo post-transfusion corrected count increments, although corrected count increments are falling somewhat into disfavor but I think they are still useful. And possibly hemostatic efficacy. So any changes that might occur as a result of this or other meetings where different plastics or plasticizers would need to be used, we have the tools to evaluate how platelets would store and whether the changes are acceptable.

The platelet assays are myriad. This is just a Whitman's Sampler of some of the major ones. There are other slides from the BEST Committee which have about 45 or 50 different tests. The fact that there are so many implies that there is no one test which gives you an in vitro evaluation of how platelets function in vivo. To do that, you still need to do radio-labeled survivals and patient transfusion studies. So any data that shows an in vitro change would have to be modified by saying, well, that is great, but what is the radio-labeled survival study show in normal volunteers and what does it do in patients once the cells are infused or the

platelets are infused.

More than the plasticizer have an effect on the platelet, the plasticizer's main effect in my opinion is on the ability of gas exchange to occur in the bag. PVC is a vapor barrier. It is a solid plastic. In order to make it flexible and malleable, plasticizers are added, and that changes gas exchange properties. And any other changes in any other kind of plastic, be it polyolefin or any other kind of plasticizer, alters gas exchange. And for platelets, that is the key. It is not the plasticizer having a good or bad effect necessarily as much as it is gas exchange, which has to occur across this container. If enough oxygen comes in for the number of platelets in the bag, aerobic metabolism through the Krebs cycle will occur resulting in CO_2 being produced, which can diffuse out of the bag maintaining proper pH. If there is insufficient oxygen because you have a bag that cannot have good gas exchange or there is too many platelets for the gas, glycolysis will occur through the Embden Meyerhoff pathway with lactic acid. Eventually the bicarbonate will be used up, the pH will fall, and the platelets will die. So the plasticizer's effect mainly, in my opinion, is for gas exchange across the wall.

The key thing is for the mitochondria to

function. That is where the Krebs cycle occurs, and if you have healthy mitochondria spewing out little green balls, everything is fine. If they switch to bad red balls or you have bad mitochondria because of lack of oxygen, the platelets will not store well. That is what needs to be evaluated. The trouble is there are not a lot of mitochondria in platelets. This is a slide from our lab where platelets were stained with JC-1, which lights up mitochondria. And then they were false stained with red to show the outside of the platelet. There is about four to five mitochondria in a platelet, as opposed to brain cells, which have hundreds of mitochondria. So what you are looking at is basically you can actually count the mitochondria in some of these. There is not very many. So any damage to the platelet that occurs from hypoxic storage would result in the potential death of the platelet.

Now plastic bag storage variables -- and I refer you to a excellent paper written by Raleigh Carmen in Transfusion Medicine Reviews in 1993, where he discusses the types of variables. Plastic sheet, and therefore bag wall thickness, surface area, type of plastic, type of plasticizer, amount of plasticizer, and permeability of the label all relate to gas exchange for platelet storage. And Raleigh and his group certainly

have done a tremendous amount of work. These are slides taken from that paper, and it lists a variety of manufacturers and plastics, some of which are not around -- some of the companies are not around. Basically, there is polyvinylchloride, which was mentioned, as a solid plastic, DEHP, which allows it to be malleable and flexible. There is the trimeletate plasticizers. Baxter had a PL732 polyolefin bag without a plasticizer and without PVC. And since this slide has been made, there have been the citrate-based plasticizers and several other types of bag, ethyl vinyl acetate and so forth. And as we get into the age of pathogen and activation as yet another net for safety of the blood supply, one would have to evaluate bags that are permeable to various types of light to see whether they would be acceptable for use in various types of photoinactivation technologies.

Now, again, from Dr. Carmen's paper, various bags which are Baxter bags and Cutter bags and Terumo bags showing oxygen transfer rate. As you can see, the PL146, which was the early plastic PVC with DEHP, only had 4 micromoles per hour. This is a Terumo bag, which is also PVC with DEHP, but it is a thinner bag and it has some other changes to allow better gas exchange. So there are ways of working around that. And then there were - these are the trimeletate plasticizers. PL1240 is

also trimeletate. This is the polyolefin bag, which was the winner at that time that this was done.

This is a slide which I did obtain from Baxter showing oxygen permeability. This is PL146, which has the DEHP and the PVC. This is a trimeletate bag. This is a citrate-based plasticizer. Here is the polyolefin. And this is another bag which is also -- it is a different type of bag that doesn't seem to have a plasticizer, PL2410. Here is yet another bag, 3014, which is a bag that has a very high amount of citrate. You really need a score card to be able to keep these in mind. But the comment that Jim Aubuchon said, the more oxygen that comes in a platelet bag, at least for now, the better. It allows you to store platelets for longer periods of time. There may be a point where oxygen toxicity may occur, but I don't know if we know anything about where that would be. And if oxygen can diffuse in, CO_2 needs to be able to diffuse out, and this is a similar type of bag. Again, this slide was obtained courtesy of Baxter.

Now this is a slide again from Dr. Carmen's paper showing oxygen transfer based on the amount of trimeletate plasticizer, which does leach out into plasma, but not to the same degree that DEHP does. And as the plasticizer content increases, it is as if you are

making more pores in the bag and more oxygen can diffuse in and CO_2 out. So, again, this was one of the comments, that the ability of a bag is necessarily based on the plasticizer, only it is the thickness of the bag and the amount of plasticizer content, and this shows this very nicely.

Now 2-DEHP, and a lot of this was shown in this classic paper by Rubin and Ness, that it is 30 to 40 percent by weight and it does migrate into plasma. DEHP, however, has been associated with some decreased platelet function in vitro. Acute toxicity is low and many other types of bags exist.

A paper by Labow in Transfusions showed that there was no specific binding site on platelet membranes for DEHP, but clearly it does bind to the membrane. About 95 percent binds to the membrane and 5 percent is in the cytosol, and it migrates into the plasma and sets up an equilibrium. If you do an SDS gel, you will see the DEHP migrating in the front of the dye as a lipid would. And the membrane bound to platelets is proportional to the amount in plasma, which you would expect. And the actual data shows that looking at the platelets over here, you can get -- in two days, you can get 19 mg/100 ml and certainly lots more, as has been reported.

Interestingly, there is a higher concentration in the platelet pellet, 37 mg/dl, as opposed to the platelet-poor plasma, only 16. But the amount recovered is much lower in the PC because there is so much more plasma than there are platelets. So the percentage of binding is greater in the plasma, although it is concentrated in the platelet. And a 5 to 10 unit pool, as Jaeger and Rubin commented on, could give you well over 114 mg of DEHP.

This is a paper by Dr. Ishikawa, where he used what is called the glow discharge technique. He took a PVC DEHP bag and treated it with radio frequency to form cross links and prevent the migration of DEHP, which was the glow discharge technology. I am not more familiar with it than that. DEHP in micrograms per ml, this is storage period. And though the control bag was leaking DEHP in its usual fashion, the glow discharge treated bag did not leach DEHP very much. So here is another possible technology. I don't know how proprietary it is, but there are ways of using the bag without necessarily having it leach in. What effect it would have on a variety of characteristics other than platelets, I am not sure.

This was a paper, again by Labow, where they showed -- they validated that most of the -- this is a

percent of C-14 DEHP. The majority of it was in the supernatant and less in the pellet, although the pellet had a higher concentration. This is percent and since there is more plasma, the number was higher in the supernatant plasma.

This is a paper by Ishikawa which shows that if you took DEHP and you incubated it with platelets over time, over 18 hours, this is the change in the ADP-induced aggregation of the DEHP-treated versus a control without DEHP. And at two hours, there was no change. The various bars show increasing concentration. This is 100, 300, and then 500 micrograms per ml. And over time of storage and with increasing concentration, the amount of ADP aggregation decreased. Now what does that mean? Well, it would mean a lot if it also meant that the platelets didn't survive very well. What it meant is that the aggregation dropped, so it dropped from 100 percent down to 60 percent. Does that give you a platelet that will still correct a bleeding time and stop somebody from bleeding despite the fact that it is somewhat less? We see aggregation studies all the time during regular storage in all kinds of bags that do drop. So I was not as impressed with this. But it still, nevertheless, points to the fact that in some in vitro systems, you can show an adverse effect of DEHP, although

not a fatal flaw, if you will.

This is another paper by Ishikawa in 1984, which shows no effect of glow discharge where the DEHP would not leach versus a control bag where the DEHP would leach on pH. But here it shows that in control bag or in a bag exposed with the methanol vehicle, there was no change in hypotonic shock response. Whereas as you use increasing amounts of DEHP, either 150 or 300, you get a drop off in the hypotonic shock response in platelets over or up to about 20 hours. We see a drop off in hypotonic shock response with platelets that are stored in polyolefin bags with no plasticizer as well. These platelets correct bleeding times. They give good corrected count increments. And not damning, but again some evidence that DEHP seems to have an adverse effect.

However, Bob Valeri, as he has always want to do, published 10 years earlier that he didn't find any changes. He stored platelets with DEHP, millimoles as opposed to micrograms, and showed that for aggregation, there was no change with collagen, ADP or epinephrine, whether the platelets were stored fresh or with varying amounts of DEHP. You could say, well, it needed to be incubated for longer period of time and perhaps so. But he, at least, found data that there wasn't a change. And also effects of addition of DEHP on platelet aggregation

to epinephrine one micromolar. Again, no change with increasing doses of DEHP versus a fresh control. So you can pick whichever study you wish.

Other studies have shown that when platelets store, they undergo the release reaction and you get a variety of microvesicles and platelet debris and pseudopods, and there is a whole scoring system that was developed. Dr. Fratantoni pointed out that platelets that are stored in polyolefin bags, however, have in addition to the kinds of pseudopod formation and so forth, as you can see in B, C, D, E, F and G, which is this paper by Labow in 1986, you see holly forms and ring forms and a variety of bizarre unclassifiable shapes. Dr. Fratantoni raised this as a question. This was data that was repeated by Labow and refers to Dr. Fratantoni's work. We don't know what this means. These were in the polyolefin bag. The survivals were acceptable. Corrected count increments were good. So what does this mean? It is not sure. Was it a lack of plasticizer? Is it oxygen? Is it something else in the polyolefin bag? Like Dr. AuBuchon mentioned, there were other things that occupied our attention and we never really pursued this. If it turns out that polyolefin becomes a much more important issue, we would need to go back and look at this again. But we do have some information. We are not

at square on. We are at square two or three.

This is a paper by Valeri again. I apologize for not putting the name in. But this was a xerox of a xerox done at the last minute before I left when I just found this paper. But this is Dr. Valeri's paper. This shows the percent of infused radioactivity, which is the radio-labeled recovery, and this is survival in days. For platelets that either are fresh or stored in DEHP plastic for 24, 48 or 72 hours. And this is believed to show that the recovery of fresh platelets is about 65 percent here. It goes down to -- this is the mean and the standard error of the mean bracketing it. About 50 percent, about 40 percent, and about 30 percent as the platelets store for up to three days. This is about what we see. We see about 40 percent plus or minus for platelets stored in any kind of a bag at about day five. That is pretty much what we see. Whether this is a plasticizer effect, unlikely. Because 732 bags give you the same results and it doesn't have a plasticizer. So when you do these studies, you have to compare storage and the storage lesion changes with what plasticizer effects might be. Regardless, all the platelets seem to have a survival of about 7 to 8 days, which would imply that of the surviving platelets -- and this is at time zero -- whatever platelets are left right after infusion

in an autologous survival model, they do survive the same length of time as fresh platelets would. So you get less recovery, but the platelets that do survive and are not damaged do circulate.

Now this was a paper by Hogge, et al. in Transfusion, which looked at corrected count increments in fresh platelets versus platelets stored after three days or seven days. And what they found was that the corrected count increment in fresh platelets after one hour was 20,000, but after three days of storage in either polyvinylchloride or 7 days in a trimeletate plasticizer, you had the same result of 10,000 to 12,000. There was no difference between these two, but there was a difference between fresh. We know fresh platelets is an anachronism. We don't have that anymore. It is merely for information. The point is that whatever changes occur, it occurs relatively frequently in the PVC bag and also in the trimeletate bag by day 7, but it doesn't seem to get any worse. So this bag is PVC with a trimeletate plasticizer, showing that we can take DEHP out and still have the same type of responses that we get. In fact, we don't use PVC with DEHP in this kind of a bag any more. Terumo does, but again they have modified it so it has better oxygen characteristics. And the 24-hour gives you the same thing at a slightly different level. So we do

have ways of evaluating changes in plastics.

This is the paper by Valeri, and all of Valeri's data came from this Environmental Health Perspectives, 1973, Volume 3, page 103. What he did -- and again, I apologize. I was trying to show you the slopes, which was all I was really interested in. This was platelets that were stored with about 20 mg/dl of DEHP, and this had about 35. This is a polyolefin plastic with very minimal DEHP, less than 1 percent. What he did was he looked at bleeding times. He gave a normal volunteer -- and it is the same volunteer in all the panels -- aspirin over here, and then he let the control go. The control is over here showing that the bleeding time went from normal up to about 12 to 14 or 16 -- it is hard for me to see -- and came down over four days to this level. The same thing here -- aspirin, control and the bleeding time goes down. When he gave platelets that were plasticized with DEHP with about 20 mg, he found that after 24 hours the bleeding time corrected after transfusion. With the polyolefin, it also corrected somewhat better. And with DEHP that had 35 mg, again the bleeding time corrected.

So what was the difference with all of the Ishikawa information showing that the aggregation studies were impaired? Well, it may be impaired but an in vivo

assay, which is the bottom line as it were, didn't seem to show in Valeri's work a problem. It corrected bleeding times whether there was DEHP, either in relatively low or higher amounts, or no DEHP. They still seemed to work. In fact, he pointed out that this was the only one at two hours that actually improved the bleeding time down to about -- I think it says 8 minutes from about 14 or so after two hours, whereas without the plasticizer, it actually took longer to get the correction.

So, again, is it helping or not? It appears that it doesn't seem to have a problem in vivo, even though in vitro it might.

Other things to be considered was a paper we published many years ago looking at 1240, which is a trimeletate plasticizer from Baxter comparing it with the trimeletate Cutter product, and this is the polyolefin. We did radio-labeled survivals in normal volunteers for platelets stored on an elliptical 1 rpm rotator, a circular 2 rpm, a circular 5 rpm, or an elliptical 6 rpm. And this had to do with the sheer stress. What we found -- this is the mean, and again it is about 40 percent recovery is what you get after five days of storage and one standard deviation. The one that lost was the PL-732 bag with the 6 rpm elliptical rotator. This is the end.

These are not days of storage down here. So what that meant was that some plasticizers or lack of plasticizer with certain types of sheer stress associated with an elliptical rotator may give you unacceptable characteristics. There is no gold standard for platelets like there is a red standard, if you will, for red cells, where you need 75 percent survival 24 hours after infusion on the last day of storage to get an acceptable red cell. For platelets, however, most people consider 40 percent recovery plus or minus one standard deviation to be a reasonable number. But the 732 and the 6 rpm elliptical rotator failed to meet that standard. All the other ones did. This was similar to -- the multiple hit survivals showed that the survivals were roughly the same regardless of the type of rotator, which was shown by the other study that was done by Valeri years ago, again, as is always the case, that those that survived circulated normally, even though fewer may have.

This classic paper by Dr. Scott Murphy and others, which basically showed that -- and this was published shortly before ours was -- this PL-732 on an elliptical rotator had an in vivo recovery of less than 40 percent, again this semi-magic number, whereas those on a tumbler did very well. Which is why we no longer -- we do not store PL-732 on elliptical rotators. In fact,

not many people use 732 very much because other bags are being used. But this kind of work shows that maybe the plasticizer in conjunction with sheer stress or the lack of plasticizer had some effect. And this would need to be looked at again further.

So the last couple of slides. Patient transfusion studies. Trimeletate plasticizer with PVC or polyolefin, looking at corrected count increments. The increments, 46,000 with the trimeletate and 58,000 with trimeletate, and 63,000 in comparable patients getting the polyolefin PL-732 without plasticizer. Corrected count increments were all in the same range. So what this shows is, again, despite in vitro studies, which may show some problems with PVC or with other types of things -- these are the trimeletates -- without a plasticizer in the polyolefin bag, you get good corrected count increments, and in vivo it appears to be acceptable.

So what are the final things we need to look at? Again, we refer to Dr. Carmen's paper. If we are going to, as a result of this conference, store platelets in some other type of bag, what the manufacturers will need to work with the public and to some degree the industry, that is, the laboratories that evaluate this, is flexibility, so that they can fill and transfer. Temperature resistance is required so you can store them

in frozen red cells or frozen plasma. The strength is required for centrifugation. Whatever new combination would have to have safety and compatibility. Various manufacturing issues, which we may here from from the manufacturers. Dr. Carmen is in the audience. And we have the ability to evaluate this and we will do it by in vitro analysis, radio-labeled survival studies, and eventually in vivo patient transfusion studies. So we have the capabilities to evaluate this. And from my perspective, we could lose PVC and we could lose the DEHP and platelets would survive very nicely in other types of bags available. The question is, are we trading the devil we know for the devil we don't know? Thank you.

DR. MONDORO: I'd like to thank the speakers very much for getting us focused on blood bags before we get into any other issues. We do have time for a short question and answer period if anyone would like to come to the microphone. I would like to remind you to state your name and affiliation for the record. Thank you.

PARTICIPANT: Herb Cullis, American Fluoroseal Corporation of Gaithersburg. I want to add to Dr. Snyder's comments that in 1998 and 1999, an additional plastic fluoroethylenepropyline was evaluated by the Phorcenias Corporation and eventually obtained approval for the storage of platelets in the United

States. It has ten times the oxygen transport of PVC plastics and six times the CO_2 transport and was found to be able to support platelets at twice the concentration of the 732 plastics.

CHAIRMAN VOSTAL: Vostal, FDA. Dr. Aubuchon, those survival studies were, I think, 35-day red cell storage. Does the DEHP beneficial effect hold up in 42-day stored red cells?

DR. AUBUCHON: I have not seen a study comparing storage of red cells in an additive system of 42 days with and without DEHP. I would think they would. I would predict that you would see the difference and I would think that red cells would not be able to be stored without DEHP for that time period, but I have not actually seen the exact comparison. Certainly at 35 days, one is not able to store red cells to meet the 75 percent criterion of 24-hour recovery without DEHP, and I don't think we would have much hope unless there is another approach, such as with the citrate plasticizer.

DR. MONDORO: I have one question for all of you if you would like to comment. One of Dr. Snyder's last point was that of temperature, and I was wondering how DEHP stacks up against alternative plasticizers with regard to the colder frozen storage of blood components as far as thawing. Is there any one that is better or

has that been -- have temperature effects been studied?

DR. SNYDER: I don't know that much about it, which of course has never stopped me from commenting in the past. But I think there is the concept of a glass transition phase in a plastic, and I do believe that some of the non-DEHP plasticized bags have better glass transition characteristics. Because that has been a problem with breakage of fresh frozen plasma, as you might imagine, during storage. So I think there are some that have improved characteristics, and that is not a major problem. If I am incorrect on this, somebody please correct me.

DR. MONDORO: Please come to the microphone, yes.

PARTICIPANT: Bob Rubin, Johns Hopkins University. I particularly liked the way the topic was introduced, I think it was by Dr. Snyder, about depending on your perspective, we've either got the hero or the villain here with DEHP. Now a large part of the evidence on the toxicity of DEHP is going to depend on in vitro studies. And I would like to emphasize this point about such studies. Some of it was reflected in these early talks. Maybe we will see more on the toxicity or toxicology presentations. And that is the nature of the solubilization of the DEHP. Now I think Dr. AuBuchon had

some data where he used sort of natural solubilization. You use a system of having a subset of plasma that you added DEHP to. Dr. Snyder, you had some data that as near as I could see used methanol as a solubilizing agent. In the Ishikawa studies, I don't think I picked up exactly how it was solubilized.

My comment, bottom line, and I would like to hear comments from the group, is the nature of the solubilization of DEHP. There are a number of critical examples where we can demonstrate either a positive effect or a negative effect of DEHP, depending on how it is solubilized. And we should keep that in mind in designing any further experiments.

DR. SNYDER: The Ishikawa also used methanol, I believe, as well.

PARTICIPANT: (Bob Rubin) If I can just follow that up and point out the major difference. Again, it may be most important in toxicology. It is using naturally solubilized DEHP, we were able to show this shocked lung or acute respiratory distress syndrome in experimental animals. In Baxter's solubilized DEHP in ethanol, not methanol, they were not able to reproduce that effect. That is the key one that I would be concerned about.

DR. SNYDER: One of the things I think we

have to be cognizant of is not only the experimental conditions for bags that are being stored, but also the effect of other external attributes, if you will, such as gamma radiation, ultraviolet radiation, effects of freezing and thawing, and even physical shaking and so forth. So when these studies are designed for future plastics, all of these various iterations and permutations would need to be taken into account, which leads you to a branch chain that can be quite labor intensive and expensive. But I think that is the challenge for the industry and for the community.

DR. AUBUCHON: Even such seemingly mundane issues as the ability to adhere a label to a plastic as it is being frozen and thawed in a waterbed.

DR. MONDORO: Any more questions or comments? Dr. Ness?

DR. NESS: Yes, I had a question actually for Dr. AuBuchon. The data you showed implied that some of the effect of DEHP in terms of red cell storage is really immediate, which led me to wonder whether anybody has looked at storing or collecting red cells in the DEHP media and then transferring them to a non-plasticized bag to see if the effect is maintained without the leaching from the bag during the storage.

DR. AUBUCHON: All of the studies that have

been reported, sort of mixed media studies, have been the other way around, where the red cells have been stored without DEHP, as you saw from the work of Tim Estep. I am not aware of anyone who has attempted that. Clearly, Gail Rock has shown that DEHP is picked up very quickly from a plastic bag. But whether over time the DEHP might diffuse to other components and the effective concentration within the red cell membrane might be inadequate to achieve these effects over time is unknown.

DR. MONDORO: We will take one last comment from Dr. Snyder.

DR. SNYDER: Yes. I would be interested as the day goes on to hear from the representatives of the pediatric community. Some of our pediatricians, for example, are still reluctant to use additive solution red cells because they are concerned about the adsol lo all these many years. So the idea of changing different plastics and plasticizers as far as the pediatric and the neonatal group, I think their comments would be extremely important in this regard.

DR. MONDORO: Thank you very much. I would like to thank the speakers. You will be seeing them on our panel at the end of the day. As I said, we have now focused your attention onto blood bags, the focus of the workshop, and our next session is going to be a more

general -- of more general interest and that will be chaired by Ron Brown.

MR. BROWN: Good morning. My name is Ron Brown. I am a toxicologist at the FDA Center for Devices and Radiological Health. As we heard in the first session, the use of DEHP as a plasticizer for blood bags clearly confers some benefits, particularly when we are talking about red blood cell storage. However, as each of these speakers this morning has eluded to, exposure of experimental animals to DEHP has been shown to have adverse or toxic effects. Those are the effects that we would like to focus on here.

I was struck by a comment that Dr. Ness had in his opening comments, particularly that some colleagues had expressed to him surprise that we thought the DEHP issue had been addressed already. I think partially that is a function of sort of the biphasic nature in which the literature has been developed. Certainly, there was considerable interest in the 1970's, largely to the work of Dr. Rubin and his colleagues, with the pioneering work on DEHP toxicity. And then it appeared in the 1980's that there was a bit of a lull in terms of the research effort that had gone on. Clearly in the past several years, there has been an explosion of research on DEHP toxicity, and we are fortunate that we

will have a number of speakers that will describe some of that research for us.

What I would like to do is to let you know that we have reordered the order of speakers in this session to allow the talks to flow more logically from one to the other. First, we are going to hear from Dr. Bucher, who is going to describe the rodent carcinogenicity studies. Then we will hear from Dr. Cunningham, who will describe the mechanisms of toxicity and carcinogenicity, particularly as they relate to the rodent studies. Then we will have a short break followed by Dr. Chapin, who will discuss the reproductive toxicity of DEHP. Then we will hear from Dr. Karle, who will discuss her recent study particularly, but in general pediatric effects of exposure to DEHP, and whether or not children and neonates represent a sensitive subpopulation. I will sort of have a catch-all talk trying to pick up on endpoints that the previous speakers had not addressed, looking at other effects produced following IV exposure to DEHP. And finally, we will hear from Dr. Ray David from Eastman Kodak on some work that the chemical industry has sponsored.

So let me introduce Dr. Bucher as our first speaker. Dr. Bucher is the Deputy Director of the Environmental Toxicology Program at the National

Institute of Environmental Health Sciences, with particular expertise in the conduct of rodent carcinogenicity studies.

DR. BUCHER: Thank you. I just walked in and discovered that we had reordered the talks. That is okay. I would like to thank Bob Chapin for running my overheads here.

I was asked to address some of the issues related to the rodent carcinogenicity studies of DEHP. There is a fairly long history of rodent studies with DEHP. There were three studies that were performed before 1982 that were considered to be inadequate evaluations by IARC when they last looked at DEHP.

The first positive studies of DEHP were the National Toxicology Program studies reported in 1982. These were of standard designs using Fisher rats and B6C3F1 mice receiving diets of up to 12,000 ppm's for rats or 6,000 ppm's for mice for 103 weeks. The doses for these studies were selected based on 13-week studies using dietary concentrations much higher or higher than that, up to 25,000 ppm's for rats and 12,500 for mice. In rats, the only real effect that limited the dose used in the chronic study was an unacceptable body weight gain at 25,000 ppm. There was also testicular atrophy seen in the 13-week studies in males at 12,500, but was not

considered to be -- would not be considered to have an impact on the chronic study. For mice, body weight gains were variable at 1,600 parts per million and higher concentrations, but they lacked a dose response.

In the rat study, as I said, the doses went up to 12,000 ppm's. Body weights at 6,000 and 12,000 ppm groups were less than controls in males and were also somewhat less than controls in females at the top dose only. Survival was pretty good in both studies, and there was, in terms of neoplastic effects, not a lot of liver effects. But there was an increase in clear cell cytoplasmic change, a slight increase in males. There was the expected testes degeneration and atrophy, especially at the top dose in males, and there was probably a related effect to this. The anterior pituitary hypertrophy probably reflecting an increased need for LH release from the anterior pituitary given the loss of testosterone feedback on the anterior pituitary.

In terms of chronic neoplastic effects in the NTP rat study, there was a modest increase in neoplastic nodules in males and females. This was statistically significant in females with a trend. There was an increase in hepatocellular carcinoma in both sexes and the combined incidence of neoplastic nodules and hepatocellular carcinomas was increased and showed a dose

response in both males and females.

At this time, the NTP declared the studies either positive or negative, and there were not the levels of evidence that we use today. These two studies were considered positive for liver tumor effects.

There were also decreases in neoplasms. There was a decrease in anterior pituitary neoplasms in males. There was a decrease in testicular interstitial cell tumors in males. And there was a decrease in mammary gland fibroadenomas in females.

In the mouse study, as I indicated the doses went up to 6,000 ppm in the feed. This was half the doses that were given to the rats in terms of dietary concentration. The 3,000 and 6,000 ppm groups had a slightly lower body weight gain than the controls in males and a little more of a body weight decrease when compared to controls in female groups in mice. Survival, again, was not too bad and not affected by treatment. In terms of non-neoplastic effects, there was an increase in testes degeneration and atrophy, although this was very slight.

The two-year study findings -- neoplastic findings in mice included a slight increase in hepatocellular adenomas in males, a mid-dose effect in females. There was more of a marked effect on

hepatocellular carcinoma in both males and females, and the combined tumor rates were increased in a dose-related fashion in males and females. Both of these studies, the male and female studies, were considered positive for carcinogenicity. And there were no decreases in neoplasms in this particular study, the mouse study.

After the 1982 studies, there were a couple of confirmatory smaller studies that were performed. Rao, et al., found an increase in hepatocellular neoplasms -- he found hepatocellular neoplasms in 11 of 14 male Fisher rats fed diets at 20,000 ppm DEHP. This is higher than the NTP doses. And that was compared to a rate of 10 percent in controls. Also at CIIT, Cattley and Popp, et al., found tumors in 6 of 20 Fisher rats, these were liver tumors, given diets containing 12,000 ppm DEHP for two years compared in zero of 18 controls.

There have been a number of more recent studies that have been reported partially. These are studies by Dr. David, who will have a chance to comment on them later. They were reported as abstracts at the SOT meetings in 1996 and 1997. These studies expanded upon the NTP studies by providing lower doses of 100, 500, 2,500 or 12,500 ppm and given to male and female rats for two years. One of their groups received 12,500 ppm for 78 weeks, and some animals were evaluated at this

time, and some of that group were held until 104 weeks to look for potential reversibility of liver tumors.

The findings of this study as reported in the abstract were that liver and kidney weights were increased and testes weights decreased at the higher doses. There were hepatocellular carcinomas increased in the 12,500 ppm groups at 78 and 104 weeks and the adenoma incidences were not reported. The NOAEL was reported for carcinogenic potential, and I presume that this includes adenomas and carcinomas, but it was determined to be a NOAEL at 500 ppm for this endpoint. And there was a statement that the tumor incidence dramatically reduced in the recovery group and that is the comparison of the adenoma and carcinoma incidents at 78 weeks as determined in similar groups of animals evaluated at 104 weeks after stopping dosing at 78 weeks.

There was also an increase in mononuclear cell leukemia in dosed males, but this was also accompanied by a low incidence in the control rate.

Eastman Kodak in 1997, I believe, also reported their two-year findings from the B6C3F1 mouse study of DEHP. Again, they used the 6,000 ppm group, which was the high dose used in the NTP study, and they went down from there down to 100 ppm. Also, the same design was used here where the high dose of 6,000 ppm was

given for 78 weeks. The dosing was stopped and an attempt of looking at the disappearance or regression of tumors was done at 104 weeks.

In this particular study, liver weights were increased and testes weights decreased at the higher doses. There is a report that hepatocellular carcinoma increased in the 1,500 and 6,000 ppm groups at 78 and 104 weeks. And, again, the adenoma incidences were not reported. The NOAEL for carcinogenic potential was, again, 500 ppm, the same as in the rat study. And the tumor incidence was reduced in the male recovery group at 104 weeks compared to that incidence at 78 weeks, but it was not reduced in the females given that same design. A reduction in liver tumor incidence in sort of a stop-study paradigm has also been seen with some other peroxisome proliferators by other folks.

There have also been some studies where DEHP has been evaluated in hamsters, and these were a quite different design. There were smaller groups of 25 male and female Syrian hamsters receiving 3 grams per kilogram by IV injection on varying weekly schedules for up to 32 weeks. Syrian hamsters were also, by the same group, exposed to air or saturated atmospheres of DEHP for a lifespan and no carcinogenic effects were reported from either study. Both of these routes of administration

bypass the gut. Therefore, the presumed MEHP metabolite and 2-ethylhexanol metabolites which are presumed to be more powerful peroxisome proliferators in DEHP would not be formed by either of these routes of administration. So it is not clear from this particular study whether the Syrian hamster is simply less sensitive to the formation of liver tumors than are rats and mice, or if in fact the proximate carcinogens, which would in this case be presumably MEHP or 2-ethylhexanol, were not formed.

There was also a study, a BASF study, reported of the metabolite 2-ethylhexanol. This was a standard design of 50 male and female Fisher rats and B6 mice. The study was done by gavage at 50 up to 500 ppm per kilogram for rats or up to 750 mg/kg for mice for 18 months. These doses were clearly high enough. Body weight deficits and increased mortality were seen at the higher doses. There was no neoplastic response reported for rats and there was no increase in hepatocellular adenoma reported in mice, but the data were not shown in the paper. There was a small increase in hepatocellular carcinomas in females, especially when compared to the historical rate in a 78-week study. Their conclusion was that 2-ethylhexanol is a weak carcinogen in female mice and may account in part for the carcinogenicity of DEHP.

In terms of genetic toxicology, DEHP is

considered negative in almost all kinds of studies evaluated. It is negative in salmonella with and without metabolic activation as are the MEHP and 2-ethylhexanol metabolites. It is negative in the mouse lymphoma assay as are the metabolites. It is negative or marginally positive in the Drosophila sex-linked recessive lethal assay. MEHP was negative in this assay. It is negative for hepatocyte or CHO cell DNA single strand breaks and UDS in in vitro studies. It is negative for unscheduled DNA synthesis in the liver in vivo in studies in rats and it is negative for DNA alkylation in rats in vivo.

There are some positive studies looking at chromosomal aberrations or induction of aneuploidy with DEHP or MEHP in fungi and mammalian cells in vitro. It appears to be negative for micronuclei formation in peripheral mouse blood in in vivo studies.

In cell transformation assays with DEHP, it seems to be positive in transformation systems using SHE cells, embryonic mouse fibroblasts, and Fisher rat embryo cells. In a paper that is important for me to mention because it is authored by my scientific director, they compared the various peroxisome proliferators with DEHP and MEHP for their ability to induce morphological transformation, chromosomal aberrations, and peroxisome proliferation in SHE cells, and there was not a clear

relationship established between these endpoints. So cell transformation may not follow directly with peroxisome proliferation.

Another group looked at the decrease that DEHP tends to give in GAP junction communication as a means of explaining the DEHP-induced transformation of SHE cells. And while it was decreased slightly, it wasn't considered sufficient to transform those cells.

There have been a number of proposed mechanisms of DEHP carcinogenesis. In most initiation promotion studies, DEHP is not an initiator, but it consistently promotes DEN-initiated altered liver foci and tumors in mice. Peroxisome proliferation is, of course, induced by DEHP metabolites, the MEHP and 2-ethylhexanol, more so in rats and mice than other species, likely through a peroxisome proliferation activated receptor alpha retinoid X receptor activation complex. This is a receptor-mediated activity. It is accompanied by liver enlargement, induction of peroxisome and microsomal fatty acid metabolism and cell turnover in the liver.

DEHP is a moderately potent inducer of peroxisomes when compared with the whole range of chemicals that induce peroxisomes. It has been shown by a number of investigators that peroxisome induction

potency does not equal cancer potency. On the other hand, studies that have been done with the PPAR, the peroxisome proliferator activated receptor, in knockout mouse treated with a Wyeth compound 14643, which is a very strong peroxisome proliferator, did not show liver tumors. So that would indicate that there is a strong involvement of the PPAR receptor in the liver tumor response.

More on proposed mechanisms of peroxisome proliferator carcinogenesis. Of course the classic idea is that peroxisome-induced oxidative damage is the cause of proliferation, although DEHP is not a positive initiating agent. It does seem to be a promoting agent. The oxidative damage there is that the peroxisomes induce enzymes that generate hydrogen peroxide more so than they induce enzymes that take care of hydrogen peroxide - catalase and other things like that -- such that there would be oxidative damage to the cell.

Kaufman at UNC and their colleagues have found that if they poison the Kupffer cells in the liver, you do not get hepatocyte proliferation when treated with DEHP. So there is apparently a role for Kupffer cell mediated mitogenic factors in this hepatocellular proliferation.

Cattley and Popp have proposed that the

promotion activity of DEHP on basophilic growth foci is stronger than on other liver foci. And it has been proposed by Roberts, et al., that they found that DEHP-treated rodent hepatocytes show an inhibition of apoptosis, and in their hands DEHP stimulates apoptosis in human hepatocytes.

Hayashi, et al., may have found at least partial explanation for the effect on apoptosis. They have found that Poly(ADP-ribose) polymerase is induced by DEHP in rodent hepatocytes. This enzyme apparently has a lot of functions, but one of them there is a requirement that this enzyme decrease for apoptosis to occur. So an induction would be an anti-apoptotic signal. There have also been proposals that the peroxisome proliferator carcinogenesis might be due to altered sex hormone metabolism. You will be hearing much more about the sex hormone effects and reproductive effects later. And there has been a proposal that it reduces serum ceruloplasm and that there might be some involvement of copper toxicity. These are much less well understood.

And I would like to finish up by pointing out that there has also been a nice paper put out recently in Critical Reviews in Toxicology that goes over the extraperoxisomal targets of peroxisome proliferators.

There are many, many extra peroxisomal targets and peroxisome proliferators. This isn't necessarily all in relation to DEHP, but there are effects on mitochondria-inducing proliferation and changes in mitochondrial enzyme activities. Succinate dehydrogenase is affected by DEHP. There are changes in microsomal enzyme activity changes in addition to those that are known with cytochrome P4504A system that is induced obviously by the peroxisome proliferators. There are changes in cytosolic enzyme activities. There are changes in hormonal pathways, and there are changes in intracellular ion homeostasis. Calcium ion, for example, is accumulated in hepatocytes treated with peroxisome proliferating agents. And there is an emerging body of evidence that would indicate there is at least the possibility that peroxisome proliferator-induced changes in a cell can lead to changes in signal transduction pathways.

So I would encourage you all to look at this reference if you are interested in alternative explanations for the peroxisome proliferation-driven hepatocyte proliferation mode of action of carcinogenesis of the peroxisome proliferators. Thank you. Any questions?

MR. BROWN: Thank you, Dr. Bucher. As you can imagine, whenever you have a compound that produces

a carcinogenic effect in rodents, there may be some significant public health or regulatory implications of those findings. I think these results have prompted a lot of research into the mechanisms by which DEHP exerts this carcinogenic effect. Dr. Bucher described some of them and we are going to hear in a little bit more detail from Dr. Michael Cunningham. Dr. Cunningham is a toxicologist at the National Institute for Environmental Health Sciences. And I think importantly, he is the team leader for the peroxisome proliferation initiative. So we are going to hear more about the mechanisms of DEHP effects.

DR. CUNNINGHAM: Thank you and good morning. I am going to restrict my comments to the mechanisms of the toxicology of phthalate acid esters in rodents and humans comparing and contrasting common features between the two species and especially in relationship to the hepatic peroxisome proliferation and hepatocarcinogenicity.

DEHP belongs to the class of chemicals referred to by Dr. Bucher as peroxisome proliferators. Peroxisome proliferators have generated extensive interest during the last 20 years. This increased interest has come about largely by the reproducible association of the induction of peroxisomes and liver tumor formation in the rodent. Since rodent

carcinogenicity is widely used as a factor in assessing human risk, there is intense interest in understanding the biochemical, cellular and molecular basis for this carcinogenic effect.

The fact that peroxisomes are induced by a large number of chemicals of various chemical classes has been used as a common mechanism to understand the basis of carcinogenicity for this class of compounds. Although as Dr. Bucher pointed out, a strict linear relationship between peroxisome proliferation and hepatocarcinogenicity has been difficult to support.

Recent data has provided focus for the hallmark effect in the rodent liver of the peroxisome proliferation phenomenon, which has been shown either not to occur or occur in a very limited extent in the livers of humans. It has also become that chemicals in this class of peroxisome proliferators vary widely in potency for this effect, from parts per million to parts per hundred.

I put this slide up to show the various examples of compounds that have been shown to produce peroxisome proliferation in rodents. Certainly many therapeutic agents that have been in the clinic for a great deal of time and have been proven safe and effective induce peroxisomes in rodents. Steroids,

herbicides, and the plasticizers that we are discussing today generally all induce peroxisome proliferation in rodents although fairly weakly compared to some of the therapeutic agents. And certainly there is a whole variety of solvents and industrial chemicals as well as food products and natural products that produce this response.

I hope you can see some of the structures. This is put up for a couple of reasons, one of which is to demonstrate the wide variety of structures that produce peroxisome proliferation from larger therapeutic type agents. Straight chain or halogenated compounds can produce this as well as some endogenous compounds such as arachidonic acid and prostaglandins have also been demonstrated to induce peroxisomes in the rodent liver.

The hallmark structural feature is that the compound has to either posses a carboxylic acid functional group or a metabolite of the compound produce a carboxylic acid functional group such as -- although DEHP does not produce a carboxylic acid group, the MEHP metabolite, which is thought to be the proximal peroxisome proliferating compound, does produce that.

In general sense, the term peroxisome proliferator denotes a drug or a xenobiotic that induces

proliferation of the cytoplasmic organelle, the peroxisome. This is an electron photomicrograph of the normal liver. Peroxisomes are constitutive in the normal liver. They are usually identified by their very dark opaque structures on an electron micrograph. Peroxisomes historically had been referred to as microbodies. Those two terms are interchangeable. These microbodies or peroxisomes are single membrane limited cytoplasmic constituents. They appear as a finely granular matrix and are ubiquitous in both plant and animal cells because they function in the intermediate metabolic pathways for the beta oxidation of fatty acids for the homeostasis of lipid metabolism.

Under conditions of peroxisome proliferation, by for instance DEHP, one can see an enormous increase in the number of peroxisomes. You can see the increase in the size as well. It may not be obvious, but the cell is also very much larger. And as Dr. Bucher pointed out, there are actually more cells in the liver. There is a combination both of hypertrophy as well as hyperplasia observed following exposure to a peroxisome proliferating agent.

The biochemical composition of peroxisomes are mainly hydrogen peroxide-generating oxidases as well as catalase, which degrades hydrogen peroxide. Often

there is an imbalance in the amount of hydrogen peroxide produced versus the amount of catalase which is present. There is also other oxidases, including alpha hydroxy acid oxidase, D-amino acid oxidase, urate oxidase, isocitrate dehydrogenase, carnintene acetyl transferase, as well as all the enzymes responsible for the beta oxidation of long chain fatty acids.

As a brief caveat, peroxisomes should not be confused with lysosomes, which contain proteolytic enzymes, acid hydrolases. They are very distinct, both in their form as well as their function.

Peroxisome proliferation has been postulated to produce an oxidative stress implicated as a possible mechanism of hepatocarcinogenicity. Peroxisome proliferators are thought to produce secondary genetic toxicity by stimulating the biosynthesis of peroxisomes, which in turn increase all these oxidase enzymes resulting in an increase or over-production of hydrogen peroxide, which is thought to react via the femptin chemistry mechanism to produce hydroxyl radical and may result then in the genetic lesions that are observed and may possibly contribute to the hepatocarcinogenicity, which is very common in long-term exposure to these class of compounds.

I think there is a great deal to learn from

the therapeutic peroxisome proliferators, and there has certainly been an enormous amount of work done with those that are used clinically, such as the fibrate hypolipidemic agents as well as the thiazolidinedione anti-diabetic agents. Much of the research that has elucidated common mechanisms has come from studies using those compounds, and I would like to use that data as a parallel for what a generic peroxisome proliferator such as the phthalates might do in rodents and contrast that to what they might do in humans.

I have already discussed all the types. This is the history of peroxisome fatty acid oxidation. You can read it as well as I can. But the point of this slide is that much of this is fairly recent. The toxicity of peroxisome proliferators is an ongoing research effort, and there is still a great deal to be learned, both on the biochemistry as well as on the toxicity of these types of compounds and certainly the relevance of peroxisome proliferation to potential adverse human health effects.

But in general, as Dr. Bucher had pointed out, the mechanism whereby a xenobiotic induces peroxisome proliferation is similar. The peroxisome proliferator in a rodent or a human has to interact with a peroxisome proliferator activated receptor in

conjunction with the RXR retinoic acid binding receptor. These two have to simultaneously bind on a response element in the gene in order to effect any transcription. In the rodent, this binding results in peroxisome proliferation. The hypertrophy and hyperplasia that I indicated before, a decrease in apoptosis, and in the rodent ultimately tumorigenesis.

Humans possess the PPAR activated receptor. Again, this is just to reiterate that the peroxisome induces hydrogen peroxide, which may interact with femptin chemistry to produce hydroxyl radical and produce DNA damage via this indirect mechanism. As stated before, there is a variety of other hypotheses, such as increase in lipid peroxidation, which may induce DNA damage by itself or membrane damage that results in lipofuscin deposition that has commonly occurred. Although this is studies for ongoing research, we have very recently generated data in our laboratory that this seems to be the predominant pathway with peroxisome proliferators inducing DNA damage, very much similar to what one would expect a hydroxyl radical type chemistry to produce and probably less likely to be through the lipid peroxidation pathway.

This slide shows the occurrence in humans of the PPAR receptor. The PPAR receptor has several

subtypes -- alpha, which is very common in the liver. Let's see, where is the liver? I can't see my own slide unfortunately. It is here. You can see the PPAR alpha content in human liver is quite significant. The PPAR gamma isoform is common in human adipose tissue. There is some reports that the levels of PPAR are significantly lower in humans and that may result in a lower sensitivity to peroxisome proliferators compared to rodents. But they do exist and are significant and are able to activate certain genes. So although they may be in lower amounts, they are certainly still active in human tissue.

There is a differential activation by fibrates which interact mainly with the PPAR alpha subtype, and so they are mainly liver active, whereas the thiazolidinedione anti-diabetic agents are thought to mainly interact with the PPAR gamma isoform and activate transcriptional events in adipose tissue more than in liver. And conversely, the clofibrate type compounds activate transcription in the liver and not in adipose tissue.

This is a schematic then of what is thought to occur upon activation of PPAR with the retinoic acid binding receptor. These bind both in human as well as in rodent at the peroxisome proliferator response element.

This is the common feature between rodents and humans. The place where they diverge then is the location of this PPARE response element to induce downstream transcription at different gene products. So even though this is common between rodents and humans, the location of this response element is key to understanding the differences in the types of gene products that are induced between the two species.

The response element has been reported in a number of laboratories either to be similar -- this is the rodent or the rat PPRE -- very similar to the human PPRE in this paper. A more recent paper demonstrated there were possible genetic polymorphisms in humans where there are actual sequence differences in the human PPRE compared to the rodent PPRE. The major common feature is that the human, both from Jan Reddy's lab as well as I think this is Ruth Robert's lab, both localize the human PPRE very much different in the relationship to the ACO co-a-oxidase and the beta oxidation gene. So that these are so far away that this is thought to explain why activation of the PPRE in humans does not result in a transcription of the ACO co-a-oxidase. Whereas in the rodents, it is very much closer and may result in the differences in the induction in the entire peroxisome proliferation response between rodents and humans.

They do have an entirely different set, then, of gene products that humans produce upon activation of the PPAR receptor and stimulation of transcription at the PPRE response element. As you can see in fibrates in the liver or thiazolidinediones in adipose tissue, instead of inducing the peroxisome proliferation response observed in rodents, they induce APO C-III gene products. They increase lipoprotein lipase activity. They increase APO-A-I and II. They both end up having lipolytic activity basically because of the lipoprotein lipase activity, and then they have their effect to decrease the triglyceride component in the plasma. Similarly to what you would see -- the end response is similar to what you would see in a rodent. But in the humans upon activation of the PPAR alpha, the transcription response is entirely different without inducing any of the peroxisome proliferation activity like you see in the rodent.

And finally, just to reiterate that and compare rodents versus humans, this is just in one gene product. Humans and rats basically do the opposite and do it through a similar mechanism. So even though we see a similar PPAR alpha expression and similar binding, the location or the response element seems to be different in rodents and humans and result in differential gene

synthesis and presumably differential toxicity. Thank you very much.

MR. BROWN: Well, thank you Dr. Cunningham. We have a 15-minute break scheduled. Because we are running a little bit late, I would like to resume this session promptly at 10:30.

(Whereupon, at 10:14 a.m., off the record until 10:33 a.m.)

MR. BROWN: Clearly, the carcinogenic effects of DEHP have taken center stage in terms of, again, both regulatory and public health considerations. But it is important to keep in mind many of the non-cancer effects that have been manifested in experimental animals following exposure to DEHP. Our next speaker, Dr. Robert Chapin, is going to address one of those endpoints, reproductive effects. Dr. Chapin is head of the Mammalian Reproductive Toxicology Center at the NIHS. And also notable for this meeting, he is part of the Center for Evaluation of Risks to Human Reproduction, which is evaluating reproductive effects of phthalate esters. So, Dr. Chapin?

DR. CHAPIN: I have been asked to give a 20-minute overview of eight-and-a-half hours worth of material, so bear with me while we start cranking here. So because of the amount of data that we have got to go

over, basically we are just going to be covering -- kind of hitting the high points, if you will.

One thing that was touched on lightly earlier is a concept that is important in this discussion of the IV exposures to DEHP and other phthalates. The diester phthalate with the two long side chains for reproductive toxicity appears to be -- metabolism appears to be required. So what happens is that esterases cleave one of those chains off and turn the diethylhexylphthalate into a monoethylhexylphthalate. Those esterases are mostly in the gut and the liver. So it is the monoesters that appear to be the active moiety. As we heard John Bucher say, when you deliver it by inhalation, it basically goes straight into the blood stream and you miss that activation step. So the internal ratio of the metabolites is different, and that would be true for IV exposure, and that is going to relate to what kind of toxicities you see for reproduction.

I wanted to just get across the point that structure relates to function. Different phthalates with different side chains will have different biological activities. Nonetheless -- and different biological activities mostly in terms of potency, which is to say that those that have shorter or longer chains than DEHP

tend to have -- tend to require more compounds to do the same kind of effect. We will see an example or we will see a manifestation of that in the next slide.

Basically, you can break reproduction down into male effects, female, male reproduction and female reproduction and the resulting fetus. So we are going to go racing through those in the body of the talk here. The male effects -- so if you are treating a pubertal or an adult male basically manifest as effects on the Sertoli cells, and I will show you an example of what that looks like. So these are sort of the mom and dad and the house, if you will, in the seminiferous epithelial, whereas the germ cells are the ones that grow up and leave. So if you affect the functioning of the hardware of the support system, then the germ cells will be adverse affected as in they die, and then that leads to testicular atrophy and reduced sperm count and reduced fertility. And we will see examples of that in just a minute.

The dose levels for that tend to be in the half to 2 gram per kilogram per day range. These are all oral studies. So what I am going to do is talk to you about oral studies, because those are the ones that, number one, where most of the data are, and number two, that is the effective route. The last three slides or so

are going to cover the couple IV -- relatively inadequate IV studies that were done much earlier, and I will just sort of address those just so that those have been covered here. But mostly what we are going to talk about are oral dosing kinds of studies.

The female effects, we tend to see reduced fertility, which manifests as a reduced proportion of females in a group of animals getting pregnant, and they have a lower litter size, and that is due to a reduced concentration of estradiol. The developmental effects -- MEHP appears to behave like an anti-androgen, but there are also changes in cell cycle, which we won't have time to go into very much.

So this is a slide from Jerry Heindel, where he was summarizing the effects of many different phthalates in a continuous breeding study, and we are going to be looking at some of the data form the DEHP continuous breeding study, and we can see that at a given dose -- at the same dose, there is a sort of increasing effect on fertility as you approach DEHP. It tends to -- and it reduces sperm concentration and it reduces testes weight. This was not evaluated, but there are changes in estrous cycle, as we will see.

So what does the testicular effect look like? Well, this is the slide that is apparently stuck

in the projector, which is a pathology slide showing the effect on the testes of a rat treated with a similar compound, dipentylphthalate, so reasonably closely related, but it produces the same kind of effect. What it finds is -- what it produces is big vacuoles in the basal part of the Sertoli cells. So we have got the seminiferous tubules in the testes, which is where spermatogenesis happens. We have got the Sertoli cells, which support those germ cells. The first structural change is -- this is sort of a testes by candlelight kind of figure. What we see here are two -- so these are the seminiferous tubules, there is one here and there is one here. This animal was treated 24 hours previously with dipenylphthalate. These two tubules look normal. So we have got basically a nice plump epithelium if you will. You can't really see it, but there are hundreds of germ cells in here with the Sertoli cells being the nearly invisible structural support in those cells. For the tubules that actually manifest the damage, you can see this basal vacuolation here. That represents an adverse response of the Sertoli cells. If you continue to dose this animal with this or any other active testicular toxicant, effective testicular toxicant, you will get testicular atrophy. The next slide shows that. Before we move on to that, I want to just for reference show you

a little arteriole in-between the two seminiferous tubules, and then here is the same arteriole. So we have gone up in power now. So now these are seminiferous tubules from an animal that has received continued treatment with a testicular toxicant, and basically all that is left are the Sertoli cells and an occasional stem cell spermatogonium. So all the germ cells are gone. This animal's testes weighs a lot less than the controls. There is no sperm here, so there is no sperm output and so there is no fertility.

So that shows you both the beginning and the end, if you will, of the testicular lesion, and that has a variety of in vivo kind of correlates. So this is the -- this is one of two slides of data that I will present from this continuous breeding study, which is basically the National Toxicology Program's version of a multigeneration reproduction study. This was done and published by Jim Lamb in the mid-1980's, and they necropsied the control group and the high dose group, so the high dose animals received .3 percent DEHP in their diet. And basically what you can see is that there was an increase in liver weight, a significant reduction in right testes weight from 135 mg to 55, and then concomitant reductions in right epididymal weight and prostate weight and sperm concentration. So sperm

concentration in the epididymis went from 473 down to 101, and in fact it would have gone down lower if we had continued dosing the animals. So significant reproductive effects there.

One of the capabilities of this design is that at the end of a certain amount of treatment in vivo, there is a possibility to cross-mate the group. So you can take the treated animals and mate them with control partners and vice versa, and you can see which sex is affected. That is what Jim did in this study. So the control/control mating, there were 18 out of 20 pairs that mated and got pregnant and they delivered an average litter size of about 8. When the treated males were mated with control females, only 4 of 20 females got pregnant and the litter size was six-and-a-half, so a little smaller but not significantly smaller than the controls. So there is a significant reduction in the proportion of pairs getting pregnant with treated males. With treated females, none of the treated females got pregnant, zero out of 16. So a clear female effect as well. So we have both male effects and female effects.

Before we move into the female, let me just summarize the results from this Lamb study. What he found was that there was reduced fertility, both at the high dose, which in this case gave an average consumption

of about 425 mgs per kg per day, and the middle dose, which gave an average consumption of about 141. And there was a clear NOAEL, no observed adverse effect level, at 14 mgs per kg per day. This is in adult mice. So the LOAEL of 141 and the NOAEL of about 14. So remember those numbers or find them in your handout, because we are going to be coming back to this later.

Okay, so you remember that we said that there was a significant female effect and that none of the treated females got pregnant. Barb Davis at the National Toxicology Program pursued that a little bit, mostly to show proof of principle and to explore likely target sites. She gave a series of regularly cycling rats a very high dose -- a high effective dose of diethylhexylphthalate. And what she found was that on the morning of proesterase, there was this estradiol surge, which then stimulates the LH surge in the late afternoon of proesterase and that stimulates ovulation and thus her receptivity and then mating happens that night. Well, in the presence of a high dose of DEHP, the estradiol surge or the estradiol rise did not happen. So without the estradiol priming the ovary, the LH surge didn't happen. And without LH surge, there is no ovulation and so there would be no -- she wouldn't come into heat.

So Barb's interpretation was that the primary effect was on the effect on estradiol here. Well, so how might that be mediated? What might be the target process that might be affected by DEHP? So what Barb did was gave -- sort of worked her way back from estradiol through the synthesis pathway. The first thing that she found was if she gave -- and as you will recall, testosterone is converted into estradiol by the enzyme aromatase. And she found that in control animals, as you give increasing amounts of testosterone, you can produce increasing amounts of estradiol. That amount is reduced in the presence of 2 grams per kilogram of DEHP. And as you went further back up the pathway, this reduction was not aggravated. So Barb's interpretation is that the primary effect is on the enzyme aromatase, which makes the final conversion from testosterone to estradiol.

So she found those effects at this relatively high dose. Then when she did the in vitro sort of dose response, she found effects occurring at this kind of concentration, which is difficult to relate to in vivo levels. But she was finding effects in the female.

Okay. So male repro/female repro development. The phthalates have been the subject of a lot of concern for the possibility that they might effect

the development of the reproductive system in developing animals, in fetuses and neonates. That puts them in the category of "endocrine disrupters" or endocrine modulators. So I need to take a two-slide sort of parenthetical, contextual setting up for you to introduce you to the concepts of endocrine disrupters so that you can put this in some kind of context.

Endocrine disrupters in general -- the concern about endocrine disrupters is that they will -- that because of in utero exposure, there will be changes in the steroid milieu of the organism or of the fetus and that will produce changes that won't happen until much later in life. And that happens because developing organ systems depend on and are very sensitive to endogenous levels of steroid. You have got to see the right amount of hormone at the right time for that tissue to say, okay, I am a rodent prostate and this is the way I am going to respond when this animal is an adult to X amount of testosterone. Or I am the rodent brain or the hypothalamus or some part of the animal. And so if you change that setting up process, then you will forever change the function and behavior, if you will, of that organ when the animal is mature. So the concept is that by interfering with this signaling process, they can change this. And the interesting thing about the

reproduction system, of course, is that that doesn't start to manifest shortly after the animal is born and you don't see it when you do a regular teratology study, which is just looking for basically the presence or absence of limbs or organs. What you are doing is you are changing the function of an organ.

For the reproduction system, of course, the function is -- that is one of the last functions to really kick in, and that only happens at puberty. So you are talking a month in mice, two months in rats, 18 years in humans. So there can be a big lag between the exposure time and the time when you can actually measure a change.

What sort of changes might you see? There are both structural and then structural changes will also lead to functional changes. But there are functional changes that lack an immediately obvious or clear, easy to find structural correlate. TCDD prevents the death of some of the cells in the middle of the vaginal folds, so you get a vaginal thread which reduces mating. So if you don't have the same amount of mating, then you get reduced fertility. You can see hypospadias compounds that behave by blocking androgen signaling to the organism will produce a series or a suite of effects, one of which is hypospadias, where the opening of the urethra

is not at the end of the penis but is someplace more closer to the body along the under side of the penis. There are smaller absent accessory organs like the prostate or the seminal vesicle. There is ectopic testes, so they don't distend into the scrotum but come out someplace in the abdomen and live between the abdominal musculature and the skin, or there are un-distended testes. There is altered anogenital distance, which in the rodent is a measure of androgen status.

Additional functional changes include altered CNS sensitivity to hormones, which would lead to disrupted ester cycles, altered libido or alterations in the ability or willingness of either the male or the female to mate and concomitant with other changes you get reduced sperm output, altered numbers of Sertoli cells, an inability to mate due to either hyperspadius or this vaginal thread, et cetera.

So this kind of sets up the kind of the context for you. Like I said, compounds that interfere with androgen signaling tend to produce a suite of effects including hypospadias and altered accessory organs and ectopic testes or distended testes.

These kind of endpoints have been evaluated for DEHP only by one investigator so far and that is Earl Gray -- or have been published by only one investigator,

and that is Earl Gray at the EPA, and he used a relatively high dose of DEHP and gave it to female rats as a part of a much larger study looking at both DEHP and like 7 or 8 other compounds.

What I will do is show you just one piece of similar kinds of data. These were data actually generated by Eve Micrease and Paul Foster at CIIT using dibutylphthalate, and what they were measuring was hypospadias. They found that there was basically no litters out of nine control litters that showed any hypospadias, but one litter out of eight, four out of seven, and two out of four showed them hypospadias at between 250 and 750 mgs per kg per day, and then this is the number of pups that evidenced that effect. So you can see a nice clear dose response relationship in the presence of hypospadias when dibutylphthalate was dosed to pregnant moms and then the kids were evaluated after birth. This is representative of the kind of data that Earl has produced, but not in any kind of dose response kind of fashion.

All right. So we don't really have the data that we really want in terms of good dose response and any kind of functional assessment for DEHP yet. That is going to change. Both Dr. David and myself are part of or running or overseeing very large multi-gen studies

that are going to be collecting these kind of endpoints. But we don't have them yet. So what have we got as a fall-back?

The next best study, I think, is one done by Arcadi, et al., where he exposed pregnant rat dames to two different dose levels of DEHP in the drinking water only during gestation and lactation. So the three-week gestation period in a rat and then the three-week lactation period and then he stopped the exposure and started evaluating the male pups at different times up to the point where they were 56 days of age, which is a little after puberty.

All of the studies that I have talked about so far have significant drawbacks from the standpoint of being able to address sort of the global issues of reproductive and developmental toxicity in rodents. The drawbacks for the Arcadi study is that the elemental/elementary kind of data collecting that they should have done was to at least measure water consumption, and they didn't do that. So we don't know how much those animals really received. Not only did they not measure water consumption, there was no assurance of how much DEHP was actually in the water that the animals received. And this is significant because DEHP is not very soluble in water, as we saw in some of

the early talks. It will go into water at very low levels, but it really helps to have lipoproteins or some sort of lipid fraction there to help haul it in. Nonetheless, if we take at face value the intended concentrations in the water and a guesstimate of how much those animals drank, then we have got a high dose of about 35 mg per kg per day, and those male pups out up to day 56 had severe spermatogenic disruption and significant adverse effects on spermatogenesis. At the lower dose level of 3 mgs per kg per day, those pups what I interpreted as delayed testes development and some disorganization, but the effects weren't nearly as severe as those produced at the higher dose level.

Okay. So that is our sort of fall-back position for the oral exposure. Let me just run quickly through two or three slides for the IV -- that covered the IV studies and looked at reproductive endpoints.

Lewandowski and Thomas in the late 1970's conducted what were then state-of-the-art, developmental toxicities studies on DEHP in rats and rabbits respectively. This is basically where you dose the mother during the period major organogenesis, and then you kill her just before she delivers and you evaluate the structure of the pups. And as we seen, there are some structural changes that are relatively easy to see, but

there are a lot of functional changes that are a lot more difficult to see and virtually impossible to see in this kind of design. And what they found was that IV administration of DEHP produced no terata, no obvious major malformations, and there were no growth effects, so there was no effect on the body weight of the fetuses. The drawbacks for these studies are only drawbacks in retrospect and with sort of the march of time and the evolution of our collective thinking. They did not examine postnatal development of the reproductive system, which is what we think -- especially if Arcadi is to be believed in toto, this development of the reproductive system may be the most sensitive group of endpoints for these kind of compounds.

Per Sjoberg, also in the late 1970's, did a series of IV administrations where he gave six IV doses, one every other day for a total of six doses of either 550 or 500 mg per kg of DEHP IV, and then killed and perfused the animals and looked at their testes under the electron microscope. At the high dose, he found relatively subtle changes in Sertoli cell pathology, only at the high dose, and no effects there. Again, he was not looking at measures of reproductive system development. So he didn't look at the urethra or the size of function of the accessory sex organs.

Then there is a series of studies from one Dr. Petersen from the early 1970's, where they gave DEHP IV in a series of six experiments. The endpoints were varied. They included terata, time to pregnancy, the percent of females that got pregnant, and as a measure of CNS development, seizure susceptibility in those pups. The main flaws with these experiments is that they were mostly fishing expeditions, I interpret, looking for flaming toxicity, if you will. Things that -- so the N in most of these experiments or most of these groups was very small. One group out of all the experiments I looked at actually had an N of 11, but all the rest of them were substantially lower. It was unclear how they performed their statistics. In one experiment, they found an increase total litter loss with an N of 4, and one of those groups again had 11. Using IV doses of 5 or 50 mg per kg administered only on gd8, which is just after implantation in a rodent. So quite rightly they thought, boy, this is a significant finding, and if this is true, it could have major impact. These are relatively low levels, and in fact we have seen these kind of levels earlier in the IV exposure to humans tox.

So they did a repeat study with N's of 11 to 16 or 18, and that study found no effect. So they were not able to reproduce the effect with a much larger study

that would presumably give us greater confidence in the veracity of the answer. So the drawbacks or the caveats to this Petersen series of studies are that they are basically very small and very few of the studies had any replicates, only this last one did. There were no statistics given. The statistical methods were unclear or not stated. And by and large, the effects that they reported were different from those found in the rest of the literature. So it is hard for me to have sort of a warm, fuzzy feeling that this is actually giving us the right picture.

So the Lewandowski and Thomas and Petersen studies suggest to me that there are little classic teratogenic potential of DEHP or MEHP, and that is comforting. But they really don't allow us any kind of firm conclusions about what the key effects might be, what the production is of the inactive parent compound to the more active metabolite after an intravenous route of exposure, and they don't tell us anything about what the circulating levels of MEHP would be there or anything about the species comparisons or, as I said, the key effects.

So let's just back up and have two slides worth of sort of final evaluation. So what we have got are the Lamb continuous breeding study, where his lowest

effect level in adult mice -- fertility in adult mice -- was 141 mgs per kg per day, and then we've got Arcadi -- and the drawbacks to the Lamb study are that they did not evaluate the development of the reproductive system in the second generation. Again, this was a state-of-the-art study at the time that it was conducted, but in retrospect it has a number of substantial drawbacks to it that limit our ability to believe that it really is founded -- that this number really is the correct number for a true LOAEL for DEHP. But the study itself -- for what they did, they did very well. And what they found was a lowest effective level of 141. Whereas Arcadi giving DEHP in the water to pregnant moms found a low effect level was his lowest dose, which was we guess about 3 mgs per kg per day.

These are substantially different. So there is a lot of room for additional data to tell us what the story really is as far as what are effective doses for altering reproduction, at least in rodent models. So from this whirlwind tour through a suboptimal data set, what we can conclude is that at higher doses, DEHP, when converted to MEHP, does affect both male and female reproduction. At lower doses, it probably affects male reproductive development and it may be behaving like an anti-androgen that is not simply behaving like an

androgen receptor blocker such as flutamide.

We are unable to conclude what an effect NOAEL or LOAEL might be at this time because of those big differences between the Lamb study and the Arcadi study, and the CERHR process, which Mike Shelby will talk about later on today, is in the process of coming to a consensus about what can we conclude from these disparate data. That process is ongoing and there is no consensus yet.

There are no good -- which is to say there are no good multi-gen studies yet on DEHP, and by good I mean studies that measure explicitly the development of the reproductive system in the second generation and measure the function of that reproductive system as well as the structure. That will change in the next year as Ray David's multi-gen study and as our multi-gen study, which we have ongoing as we speak, come to completion and get reported out.

So it has been my job to talk and yours to listen, and I hope we finished at the same time. Thank you very much.

MR. BROWN: Thank you, Dr. Chapin. Our next speaker is Virginia Karle. Dr. Karle is an assistant professor in pediatrics at the Department of Neonatology in the University of Alabama at Birmingham. She also

serves as the medical director of the Neonatal Intensive Care Unit at Medical Center East in Birmingham, Alabama. Dr. Karle?

DR. KARLE: Thank you. First of all, I have eliminated some of the slides from your outline because of the issue of time.

When we look at the issue of pediatric toxicology and phthalates, we have some special concerns when we look at the pediatric population. First of all, the data is very limited to about a half a dozen studies in the literature. This is primarily looking at newborns and there are a small number of patients in each of these studies. We have to keep in mind that these are critically ill infants who are exposed to a variety of devices and procedures putting them at risk. Their immature metabolic pathways may also potentially put them in a subpopulation that makes them at greater risk for toxicity.

In the literature, we have seen that DEHP exposure through a variety of procedures have been reported. Looking at blood product transfusions, umbilical catheters, exchange transfusion, cardiopulmonary bypass for corrective heart surgery, mechanical ventilation and long-term bypass such as ECMO.

Hillman, et al., reported in 1975 that DEHP

could be extracted from heart tissue in 17 neonates. She compared neonates who had been had lines or had been transfused and compared those to stillbirths that were not exposed to these procedures. They found higher levels if the infants had an increased number of transfusions, if they had an increased number of line usage, or if they had died early. She also noted that in the more premature infants who died three to five months after their exposure, they could detect tissue levels at this time. In addition, they reported three neonates who died of necrotizing intercolitis and found gut tissue levels in these infants. A cause and effect relationship could not be determined.

On this slide, I have combined two studies looking at double volume exchange transfusions, a study by Sjoberg reported in Transfusion in 1985, and Plonait from Transfusion in 1993. These are the number of patients, 6 and 16. These were all newborns who underwent double volume exchange transfusions because of ABO incompatibility and RH isoimmunization. The amount of DEHP measured in the blood bags used for the exchange ranged from 36 to almost 85 micrograms per ml in this study to 4 to 123 micrograms per ml in Plonait's study. But the actual amount measured in the patients at the end of exchange ranged from as little as 3.4 to as much as 21

micrograms per ml.

In each study, they noted whether there was any accumulation in babies if they had repeated exchanges. In Sjoberg, he reported no accumulation over time. But Plonait did report that if an infant was repeatedly exchanged, their baseline value did increase over time. They also noted the clearance of DEHP from plasma levels, and noted in both studies that the more immature or premature the infant and the number of repeated exchanges resulted in a decreased clearance of this compound from the blood. Plonait also went on to state that looking for evidence of clinical toxicity, there was no signs of cholestasis or cardiac dysfunction in these babies looking at indirect measures -- heart rate and blood pressure.

Berry, et al., looked at DEHP exposure from short-term bypass in adults and infants who had corrective heart surgery. They measured both DEHP and MEHP levels pre and post-bypass and saw a 7 to 10-fold rise at each by the end of their bypass run for surgery. He reported that infants had the highest level at a range of 5.1 microgram per ml for DEHP and 2.7 for MEHP. They noted that most of the levels decreased and dropped to preoperative values by 24 hours except if they had decreased urine clearance, and then levels may persist

for as long as four days.

As a neonatologist, my interest really concerned this subject when it came to the ECMO baby. These are newborn term babies that are put on this device for oxygenation reasons. In addition to this circuit, which is filled with blood at the initiation of bypass, they have ongoing transfusions and their blood circulates through this tubing for periods of days to weeks on time at temperatures of 37 degrees centigrade, putting this population of baby at greatest risk for acute exposure.

Schneider, et al., first reported the exposure from DEHP in the ECMO patient through a letter in The New England Journal of Medicine in 1989. They reported one patient who had levels after 14 and 24 days of bypass in the range of 26 and 33 micrograms per ml. In addition, they looked at tissue levels in an autopsy patient who had died of respiratory failure and detected liver, heart and testicle levels of DEHP. To look at the potential exposure from the ECMO circuits themselves, they also ran two circuits for a period of 48 and 84 hours and measured extraction or leaching of the DEHP over time in a range of 3.4 micrograms per ml per hour and 4.1. They took this number and they extrapolated that to the average 4 kg patient who would be on bypass for 3 to 10 days and projected that they could

potentially expose a baby to these levels, 42 to 140 mg per kg body weight, in that time frame. This obviously is much higher than has ever been reported in patients.

My studies and I also looked at ECMO circuits and wanted to look at the design effect in its role. We compared three ECMO circuits that were clinically used at the time. Circuit A is what we use in our institution at Children's National Medical Center. Circuit A had a smaller surface area of 932 ml centimeters, a volume of 800 cc. Circuit B was a larger circuit used in some centers. This should be 1,000 mls. And circuit C is the actual same as A, except for the internal lumen has covalently bonded heparin. These circuits were primed in the usual fashion with saline, albumin and packed cells. We also added CPDA solution because of hemolysis and clot formation. We circulated these at 400 cc a minute for 48 hours and corrected the blood for physiologic pH.

The amount of DEHP at the end of the prime or time zero ranged in the circuits from 18 to 21 micrograms per ml, which is similar to that reported from blood bags for exchange transfusions. The extraction rates over time for the smaller circuit was at .32 micrograms per ml, just 10 times less than what Schneider

had reported. Circuit B, which is larger than this is volume, was almost twice the extraction rate. And circuit C actually had decreasing amounts of DEHP extracted over time.

This figure represents percent change from baseline over time, 0 when the blood has been added, at one hour, and every six hours for a 48-hour time period. With circuit A, what we use in our institution, we see a rise over time for an extraction from the ECMO circuit itself. For the larger volume circuit, this is increased. But for the heparin-bonded circuit on the internal lumen, we see a disappearance or a decreased amount of DEHP measured over time. Represented in this fashion with DEHP concentration corrected for surface area, we see that there is no difference between A and B when you account for the surface area. And again C disappears over time, which is consistent with what we know for DEHP metabolism in plasma to its by-product, in particular MEHP.

We concluded from this part of our study that DEHP does leach from ECMO circuits and that the design of the circuits, such as tubing type, size, length is important for the amount that could be extracted. And that the Carmeda heparin bonding circuit on the internal lumen may actually be protective.

If we look at the literature and what has been published as to exposures from medical devices, we see that from various procedures we have extrapolated this to a 4 kg patient in terms of milligrams per kilo. From whole blood transfusions, depending on who you read, it is on average about a half a milligram per kilogram of body weight for a single transfusion of 10 cc per kilo. For platelet transfusions, it is higher at 1.9 milligrams per kilo. For dialysis, 1.9. For double volume exchange transfusion, it can range from .8 to 3.3 mg per kilo. When you compare that to the ECMO patient, this estimates a potential -- circuit A for a three-day course of ECMO, 4.7 up to 15, and for the larger circuit as much as 35, and for the Carmeda circuit itself zero -- compared to Schneider's study, which they estimated ranges from 42 to 140 mg.

We see in the literature that the patients are exposed through these devices, but what evidence is there that there is toxicity? Schneider and his colleagues also reported an association between cholestasis in the babies on ECMO, and they looked at hemolysis and DEHP levels as factors for this cholestasis. They measured in 29 ECMO infants DEHP levels at 48 hours before the end of bypass or decannulation. They also measured bilirubin levels and

free hemoglobin as a measure of hemolysis. They defined cholestasis as mild if the direct bilirubin was less than 1 mg per dl, severe if it was greater than 2 or 80 percent of the total, and moderate for everything in-between. The amount of DEHP levels reported in their patients ranged from 18 to 98 micrograms per ml.

They noted that they did indeed find cholestasis in the infants on ECMO and saw high direct bilirubin levels without other evidence for cannicular or hepatocellular injury. The transaminase levels were normal. They did note the DEHP did not correlate with time on bypass. DEHP levels, hemolysis and the need for ultrafiltration did correlate with cholestasis.

They looked at relationship between DEHP levels and hemolysis and stated that it correlated with an R of .67, and speculated that DEHP may actually cause hemolysis and instead of stabilizing the red cell membrane may actually cause hemolysis or at the very least prevent excretion of bilirubin from these patients.

My colleagues and I at Children's National Medical Center in working with Dr. Rubin at Johns Hopkins looked at this issue and wanted to look at the issue of toxicity as well. We looked at plasma levels collected in glass and stored at minus-70 degrees until analysis was done by gas chromatography. We looked at term

infants with respiratory failure and had minimal requirements of 100 percent oxygen and peak pressures of 30. Those babies that met institutional criteria for ECMO went on to bypass. The others were considered controls. We had 18 ECMO babies and 10 controls.

The clinical signs of toxicity that we evaluated were the lung by looking at a chest x-ray scoring system, the liver looking at bilirubin total and direct, cholesterol, triglyceride and transaminase levels, and heart function by measuring cardiac echoes. We measured daily DEHP levels before bypass and after for three days after they were decannulated in the ECMO babies and daily until the control babies were extubated.

There were no differences in demographics between the patients except for in the respiratory parameters, where as expected the higher respiratory settings were in the babies that went on to ECMO and the lower oxygenation parameters, and thus the sicker infants went on to bypass.

In our study in 18 ECMO infants, before bypass we detected no DEHP in the blood. We were going to compare that to mg per kilo weights so we can look at the previous literature. After one hour of bypass, the mean level was only 3.5 micrograms per ml or .8

milligrams per kilogram. After three days of bypass, the mean level was 4.9 or 1.2 milligrams per kilo. In the highest level per patient, the mean value was 8.3 micrograms per ml or 2 mg per kilo, similar for that seen with transfusions.

At decannulation or at the end of bypass, the levels had fallen and not accumulated to levels of 1.3. On this figure, we see DEHP concentration over time, and this represents all DEHP levels measured in the 18 ECMO babies. This part of the graph is the N or number, and this represents the percentage of non-detectable DEHP levels in these patients. Again, before bypass 100 percent of the babies had no detectable levels. But even at one hour of bypass, a third of the babies had non-detectable levels of DEHP. Most of the values ranged under 12 micrograms per ml except for one patient, and further out on bypass, 9 to 10 days, 100 percent of the babies had non-detectable levels.

We also found that there was no DEHP in our non-ECMO or control patients except for one baby that had a level of 5.1 who had just been transfused. In our study, we tried to avoid transfusions or recorded the timing of that between that and when the levels were collected.

Again, the DEHP levels ranged from zero to

24. In two babies, they had circuit changes and the DEHP levels rose briefly and then decreased. When we looked at analysis between DEHP levels and our clinical signs of toxicity, we saw no correlation when looking at heart, liver or lung parameters. In particular in the liver, we saw no group differences in liver function between the ECMO and non-ECMO patients, nor did we see any evidence of clinical significance or cholestasis, which conflicted with Schneider's study. In the heart data, we saw changes in heart function consistent with that which had been previously reported in the literature for babies and adults on bypass, but again these numbers did not correlate with DEHP levels.

We were particularly interested in looking at lung in looking for evidence of toxicity because of this evidence of shock lung or white out reported in animal studies, seen in patients after cardiac surgery on bypass, and a white out phenomenon that is noted in the ECMO babies within 12 hours of cannulation. We know that this white out is associated with surfactant protein A production and a decrease in that. We evaluated this by looking at a chest x-ray scoring system that had been initially developed for the premature baby in evaluating RDS and then adapted to the ECMO population.

The highest score in the lung data was at

the beginning of ECMO in the ECMO patients, correlating with their acute illness. But again, the levels did not correlate with DEHP levels. This figure shows the lung data. Chest x-ray score ranged from 4 to 20 with ECMO babies shown in blue and controls in white. So the levels were higher at the beginning of bypass and then decreased. This was statistically different than the control patients, but again did not correlate with DEHP levels.

We concluded from our study that ECMO does expose these patients, but levels are lower than previously reported. The risk from the circuits in our study was 4.7 to 35 mg per kilo depending on the length of time on bypass. However, measured in the patient it was actually in the range of 2 mg per kilo. We propose that differences in circuit design and content of plasticizer in those circuits and transfusion practices may account for the differences between ours and in particular Schneider's studies. We found no evidence for toxicity in these patients when looking at lung, liver or heart parameters.

In summary, DEHP is detected in newborns after exposure from a variety of medical devices, but evidence for acute toxicity has not been shown in this population. Thank you.

MR. BROWN: Thank you, Dr. Karle. I am going to ask Dr. Vostal -- or Traci. Many of the studies that we have heard described this morning use oral dosing. Again, several of the speakers had pointed out that DEHP is converted to its presumed toxic metabolite, MEHP, largely in the gut through the action of hydrolases. The challenge to us as a regulatory agency in evaluating that data is how do we make sense of the oral toxicity data and how do we use that to assess the risk posed by patients exposed to DEHP and MEHP parenterally. Those are the issues that I would like to touch on.

One way that would allow us to use the oral data is to do a route-to-route extrapolation of dose or potency. I want to discuss some issues related to route-to-route extrapolation. But more importantly, if we are going to do this risk assessment for patients exposed parenterally, what parenteral data do we have.

We have heard a little bit from the speakers, in particular Dr. Chapin, in terms of the available IV reproductive toxicity studies. But I would like to touch base on a couple of other endpoints and share with you our thinking as we go through our risk assessment in the Center for Devices and Radiological Health. Also, what factors are we considering as we

evaluate these studies for use in risk assessment?

In addition, what I would like to do is to try to put these exposures and animal toxicity results into perspective in terms of how do the NOAEL's and LOAEL's that we see in the animal toxicity studies compare to the doses that patients are getting clinically. I won't go into a lot with this. I am really going to focus on patients that are transfused. But I think this will at least give us a perspective on where the animal studies fall out relative to what patients are getting.

Now I am going to refer any real discussions of clinical relevance to the clinicians, especially during the question and answer period. But I am probably going to raise more questions than I answer in terms of clinical relevance. But I do want to point out that patients that are exposed to DEHP through transfusion scenarios have adverse effects that are very similar to those that we see in the experimental animal studies. And I think those are going to raise some questions about the potential role of DEHP and the pathogenesis of these adverse effects in patients who are transfused.

Again, I mentioned and other speakers have said that DEHP is converted to its presumed toxic metabolite, MEHP, in the gut. But I think it is

important to keep in mind that we do have esterases in the liver that have the ability to convert DEHP to MEHP. So that doesn't totally negate all the oral toxicity studies. We also note that -- and I think it had been pointed out before that Dr. Rock has shown that plasma has the ability to convert DEHP to MEHP during storage. So we can't totally discount toxicity occurring via a parenteral route of exposure.

Unfortunately, I am not aware of any studies that have looked at the toxicity of DEHP in parallel following oral and intravenous administration. The closest that I could come was the study that looked at the relative potency following and oral and IP administration. This was the Shiota and Mima study in which they had administered DEHP to pregnant ICR mice on days 7, 8 and 9 of gestation. In the study, they found teratogenic effects at doses greater than or equal to a gram per kilogram, again a high dose. But I think it is notable that following intraperitoneal administration that there were no effects at doses up to 8 grams per kilogram. So clearly there is a route difference here. I am not sure how much we can extrapolate to the IV administration route, but showing the difference between parenteral and oral administration, there is a difference in potency.

What are the practical implications then if we know that there is a difference in potency between oral and parenteral administration? I think at a first level that whenever you are trying to set a tolerable level for DEHP or even looking at a margin of exposure analysis, I think you have to go about it with caution in trying to use the results of the oral toxicity data. And at this point, we would recommend not using those data unless we had a means to conduct that route-to-route extrapolation. Either a physiologically-based pharmacokinetic model or other approach that would allow us to do that. So at least for right now our early thinking is we are going to stick to the IV data or the IP data in trying to assess the risk of patient exposure to this compound.

Dr. Chapin had very eloquently given an overview of the reproductive toxicity studies and had mentioned the reproductive tox studies, including all their flaws and warts. What I would like to do is briefly describe other endpoints that have been seen following intravenous exposure of experimental animals to DEHP and MEHP.

In the 1970's, and I think we had heard other speakers mention this, it was recognized that patients that are receiving massive transfusions would

develop adult respiratory distress syndrome. And it is curious that in experimental animal studies, we are seeing very much the same histopathology that we see in these patients that are getting large volume transfusions. One of the early investigators that called this to our attention was Bennett, who showed that with stored blood we were seeing adverse pulmonary effects in baboons in a whole range of endpoints -- vascular resistance, end expiratory pressure, PO_2 gradients. With stored blood, you see adverse effects in all of these endpoints. It is important to point out, though, in the Bennett study that they did not document the type of bags that the blood was stored in. So we can't necessarily implicate DEHP as the causative factor here. But I think this study certainly led many investigators to think that DEHP might be involved in the etiology of adverse pulmonary effects.

Bennett and colleagues originally had attributed these effects seen in the earlier study to the generation of micro-aggregates. I think in a following study they had shown that that was not necessarily the case.

I find myself at somewhat of an awkward position describing the results of studies done by participants sitting here in the room. So I would

encourage Dr. Rubin, in particular, and Dr. Jacobson, if you have any comments on the remarks that I make, please raise those in the question and answer period. But I wanted to point out that it was the early work that Dr. Rubin had done in experimental animals -- and we had heard some of that -- that had raised the suggestion that intravenous exposure to DEHP could cause adverse pulmonary effects in these animals. And in patients undergoing cardiopulmonary bypass or transfusion, we are seeing increased levels of DEHP in the lung tissue of these patients.

I think in an earlier question and answer period, Dr. Rubin had hit really on one of the key aspects that I think is important to consider as you evaluate these studies. And that is the physical state of the DEHP or how it is solubilized. Is it naturally solubilized by leaching from the PVC bag into the blood or blood product, or is it solubilized in an exogenous surfactant? Those factors potentially are going to have an effect on the manifestation of toxicity. In one of his early studies, I think he had shown the effect of tween used as a surfactant in the manifestation of toxicity. When DEHP was solubilized in tween, we find a range of adverse pulmonary effects -- respiratory distress, increased lung weight, hemorrhagic effects --

when DEHP was solubilized in tween or tween and DMSO.

Looking at just the control, there were essentially no effects. But it is important to note that when DEHP was solubilized in BSA or acacia, how you are also not seeing effects. I don't think this necessarily negates the potential for naturally solubilized DEHP to cause pulmonary effects. But I think it does show a potentiating factor of the surfactant, and that just needs to be taken into account. In other words, I don't think we can discount these studies, but I think we need to look very carefully at how the DEHP was solubilized.

This is an unpublished study. This was done as an NIH contract by Rutter, et al., in which they administered varying doses of DEHP administered neat to dogs for a four-week period over six days a week. This was done intravenously. And they had shown at their lowest dose, which was 25 milligram per kilogram just time averaged for six days a week is around 21, they were finding increased lung and liver weights in these dogs. There really was not a lot of histopathology done, but this was one of the -- there was clinical chemistry done, but they did notice the increased lung weight. Again notable because this was DEHP that was not necessarily solubilized in a surfactant but administered neat.

It is also interesting to note that in a

follow-up study, Rutter and colleagues had taken DEHP that was solubilized from the PVC bag -- and naturally you are not going to be able to get as much DEHP into solution, so the DEHP doses are going to be much smaller. But here in a situation that would mimic storage of blood or blood products, you are getting less DEHP solubilized into the blood or blood product, and you are also getting no adverse effects noted. In a similar study -- this again was done in dogs -- in a similar study in rats, Garvin -- and this was published in an abstract only -- noticed no adverse effects in pregnant rats with a wide variety of endpoints, but in particular pulmonary effects, at doses up to 3.7 milligram per kilogram per day. So one of the key factors here may be the state in which DEHP is solubilized and how the effects are manifest.

This to me is a very intriguing study because experimental design mimics a clinical situation that would parallel one in which a patient would get large volumes of blood transfusion. That would be a trauma patient or perhaps one that was hypovolemic -- this was done by Dr. Rubin and colleagues -- in which they had sonicated DEHP in plasma and then added it back to the packed cells to reconstitute the hematocrit. And I think what is notable here is that Dr. Rubin had shown

that the distribution of DEHP in the plasma and blood was similar to that that you would find if the DEHP had just been leached out of the PVC.

The experimental design consisted of two phases. One in which the rats were bled and then retransfused at the same time in an exchange transfusion. The other aspect of the studies is where the rats were bled out and kept hypotensive and hypovolemic for a fixed period -- and I believe 30 minutes -- and then retransfused. And you find that even at relatively high doses -- now they didn't report a LOAEL, but LD-50 is up around 200 mg per kilogram per day, so a relatively high dose. But in this situation where the rats were made hypovolemic and held that way, the LOAEL dropped dramatically, on the order of 8 to 13 milligram per kilogram per day.

Again, I think this is interesting for two reasons. One, that they had taken great care to look at the partitioning of DEHP in the blood and found that it was similar to that that you would expect if the DEHP had just leached from the bag. And also, this situation in experimental animals that mimics what we might find in a clinical setting.

Again, these are very preliminary conclusions. But the effects that we see after large

dose IV injection of DEHP that has been solubilized in aqueous media or serum or some surfactant. We tend to get greater manifestation of toxicity, pulmonary toxicity, than when we see if the DEHP just leaches out of the PVC bag at clinically relevant doses. And again this is very preliminary, because I know there are some questions about this study, but the LOAEL for pulmonary effects in experimental animals appears to be on the order of 8 mg per kilogram per day. And as Dr. Chapin showed, and we will come back to that when we start to look at the doses that patients are exposed to in various clinical scenarios.

Dr. Karle had discussed to some degree some of the cardiovascular effects. We are limited in that most of the studies that we have are either in vitro or ex vivo studies using profused lung -- or profused heart preparations. So we are limited in the extrapolation to the in vivo state. But I think these studies may be relevant and at least in a hazard identification perspective. When we look at Dr. Rubin's early work, he had shown that a dose of 4 microgram per ml was lethal to embryonic chick heart cells, suggesting that there is some cardiotoxicity from DEHP. I have to confess I don't know how this was solubilized, but Peterson looked at injection of neat DEHP to dogs had found -- excuse me,

this was a profused rat heart preparation, the Peterson work rather than work in dogs. This was their LOAEL, 500 microgram per ml, and they were finding effects initially with an increase in heart rate and then a decrease. And as the preparation was allowed to proceed, then eventually a decrease in the amplitude. So a negative inotropic effect of DEHP solubilized.

Again, Dr. Karle had mentioned the Berry study. This was an profused trabecular muscle in vitro in which there were negative inotropic effects across this dose range.

How does this translate to the clinical situation? Unfortunately, to my knowledge we don't have good IV rodent studies that have looked at a range of cardiovascular effects. When we look at the clinical studies -- when we look at Dr. Karle's studies, and we just heard her mention that there was no evidence of cardiotoxicity in these neonates, and the endpoint that she had looked at was echocardiographically. When we look at the Plonait study that she had also mentioned, there is no change in heart rate. Again, relative to the focus of this meeting, this was in patients who were transfused. No change in systolic or diastolic blood pressure within 24 hours after exchange transfusion in neonates. So although there is a hint of adverse

cardiovascular effects that we see in the in vitro and the ex vivo studies, we aren't necessarily seeing those translated into the clinical setting.

Hepatic effects really are the hallmark of DEHP toxicity following oral exposure in rodents. So a key question would be are we seeing those effects following intravenous administration to either experimental animals or humans? And here the data, like so many endpoints, are very mixed. Unfortunately, we can't say with any certainty that, yes, we are seeing hepatotoxicity following intravenous exposure. And again, just like so many other endpoints, we are handicapped by the limited number of studies that are available.

This was a study of Greener, et al., in which they administered DEHP intravenously to three-day-old rats every other day, and they saw slightly increased liver weight and SGOT levels, but again the doses were very high. This was a very short-term study.

The Rutter study that I mentioned earlier, the intravenous study in dogs, they had also seen slightly increased liver weight with a LOAEL of 21 mg per kg per day, notable, I think, because it is a non-rodent study.

At least from our perspective at the Center

for Devices, this study that was conducted by Jacobson really represents a key study. This was one in which monkeys were chronically transfused with platelets or plasma stored in PVC bags for various periods. Controls would receive platelets or plasma stored in polyethylene bags. There were a range of subtle hepatic effects observed in monkeys that have received these blood products in PVC, including abnormal liver/spleen scan ratios, abnormal BSP clearance and altered histopathology, which I think is notable and was also observed in six out of the seven animals which received the DEHP.

The Jacobson study is strong for a number of reasons, one of which we had heard about one of the limitations of the Arcadi study was that they didn't quantify the levels of DEHP in the drinking water in that study. Here we are fortunate and they were very meticulous to have quantified levels of DEHP in the plasma that the monkeys had received or the platelets, and were able to identify with some certainty what the doses are. Now these are means for the various animals in the different exposure groups.

It is also interesting that in addition to conducting a toxicity study, they had looked at the dose of DEHP received by patients receiving transfusions on a

chronic basis that either had aplastic anemia or leukemia. We note that the doses received by these patients over a year fall essentially within the range of doses that we were seeing adverse effects in monkeys that were chronically transfused.

Now what are the reasons why this may be useful to us as a regulatory agency in assessing the risk posed by patient exposure to DEHP? Some of the strengths of the study are I had mentioned our limitations, and we have so many oral studies and so few IV studies. Here is an IV study that gives us some very interesting data. The clinically relevant route of exposure. We are not worried about those earlier concerns about how is the DEHP solubilized before it is administered. We have got chronic long-term exposure, which is important. We don't have to worry about effects at an MTD or a high dose. These are all clinically relevant doses. And important, we don't have to worry about many of the concerns that have been raised about effects manifested in rodents, particularly as they relate to mechanisms regarding peroxisome proliferation. So we have got a primate model here. However, there are some concerns that have been raised about the study and hopefully we will be able to discuss these more in the question and answer period.

The authors point out that there was a

tuberculosis outbreak in this colony of monkeys and that it is impossible or it is difficult to discount this as a confounding factor in the hepatic effects that were seen. The plasma was pooled and then retransfused to the monkeys, so the potential exists for a reaction to foreign protein in the pooled plasma. One limitation that we always have in using primates is the small sample size. This is really going to limit us from doing a statistical analysis. But more importantly, I think it draws into question some of the effects that were seen. And many of the endpoints that were assessed were subtle effects in endpoints that may not be usually assessed in a patient population. They wouldn't be liver enzymes, for example, exclusively.

So where do these NOAEL's and LOAEL's fall out? We saw in the Garvin study and the Rutter study in which DEHP was allowed to leach from the PVC bags, we are seeing no effects at around 1 mg per kilogram per day. And here in these clinical studies, we are seeing no effects at doses somewhat higher. Dr. Karle, I just put a question mark here, because I just took your dose from that circuit B and assumed that occurred over three days. So this is like a worst case.

Dr. Karle had mentioned the Schneider study when looking at hepatic effects. There is another study

by Ganin, in which they had reported that there was an increased level of peroxisomes in patients that had undergone hemodialysis, but I think there are a number of concerns about that study, and I haven't raised that as evidence of hepatic toxicity in humans exposed to DEHP. But we do have this study that Dr. Karle had mentioned, the Schneider study, in which they had seen cholestasis in patients on ECMO. But again, to counter that, there were no hepatic effects seen in the Plonait or the Karle studies.

Again, in trying to put these effects and the dose at which the effects occur into some perspective, one way to do that is to just look at a margin of exposure. And simply that is what effects do we see adverse effects in experimental animals or humans, and how does that compare to the dose that humans are getting in this case in clinical scenarios. And I want to point out that this is not a risk assessment. Because we are not attempting to characterize the risk posed by exposure of patients to given doses of DEHP. This is really more of a qualitative evaluation comparison, if you will. And at least we think that this comparison is only valid if you compare effects and doses that occur across the same routes of exposure and durations of exposure, because of the concerns about route-to-route

extrapolation of these effects.

And when we do that, what do we see? Again, I have to mention that these comparisons are very preliminary, and they are all worst case. We assume that one of the lowest doses that produces adverse effects in experimental animals following intravenous exposure was seen in Dr. Rubin's study, and the LOAEL for that was about 8 mg per kilogram per day.

Dr. Chapin had mentioned the Petersen study, in particular the shortcomings of the Petersen study. So, again, we have some questions here. He had also mentioned the LOAEL for the Sjoberg study. This is just the 500 divided by two, because the dosing was every other day.

The unpublished Rutter study, we were seeing pulmonary and hepatic effects at a LOAEL of around 20 mg per kg per day. And again here, we see the disparity with adverse effects in the Jacobson study down three orders of magnitude less.

We have a handful of studies that have given us information on doses of DEHP and MEHP in patients that are being transfused. We can expand this if we consider hemodialysis and ECMO. But at this point, we will just consider doses received by patients undergoing transfusion. And they may be on the order between 1.8

and 4, and the Plonait study, as Dr. Karle had mentioned, they may have doses up to 22 and 23 mg per kg per day.

Dr. Rubin earlier had shown for trauma patients that received large transfusions, we may be up on the order of 8 mg per kg per day. And in a more chronic transfusion scenario in patients with aplastic anemia or leukemia, we are talking about doses somewhat less when time averaged over a long-term period.

One of the discussions that I hope we can foster in the discussion period is, again, these studies are somewhat older and in the discussion period at the end of the meeting, we have invited a number of clinicians that hopefully will share how the clinical practice of medicine has changed, if it has, to affect these dose estimates. Because we recognize that these may not be the most contemporary or accurate estimates that we have at this time. And DEHP exposure may have changed in the course of time.

So how are the doses that patients are getting compared to doses that are producing adverse effects in animals? Well, again, if we assume that this 8 mg per kg per day from Dr. Rubin's work represents a LOAEL, and if at a worst case we take data from the Plonait study or -- I am sorry, this is Plonait -- and Sjoberg, we are finding margins of exposure that are

fairly close to our LOAEL's that we seen in experimental animal studies. If we look here at the estimate of dose that Dr. Rubin had offered for adult trauma patients, we are about one for margin of exposure. In other words, patients are being exposed potentially to DEHP and MEHP at levels that may have produced adverse effects in experimental animals. Those are short-term exposure scenarios.

In longer term, we either have a LOAEL from the Rutter study compared to doses that Dr. Jacobson had found -- in this case, we see a margin of exposure that is considerable, which would lessen our concern about the manifestation of adverse effects in these patients from DEHP. But you can see why the Jacobson study is so key to our assessment of patient risk, in that if we are really seeing adverse effects in a primate model here at these very low doses, that we may have some concern for the manifestation of these effects in patients if we looked at very sensitive endpoints.

Another way to look at margin of exposure is not necessarily dose as a mg per kg per day basis, but it would be on concentration. Again, Dr. Karle had mentioned the Berry study in which we were seeing -- and also the Rock study in which we were seeing effects here at 15 microgram per ml. In the Sjoberg study they had

measured levels of MEHP -- excuse me, this is MEHP -- around the same levels. So, again, the potential exists from ex vivo and in vitro studies for the manifestation of cardiovascular effects. But again, in the limited number of clinical studies that have been conducted, we are not really seeing these in the patient population.

I have two slides here to just sum up. I have not put conclusion slides, because again we are still going through the process of assessing the risk of exposure. But what are some of the challenges that we face as a regulatory agency in assessing these risks? Notable among them our interpretation of the study. Again, I mentioned the Jacobson study and the Rubin and Chang study. How much confidence can we have in the adverse effects that are seen in experimental animals, and can we really use those in assessing the risks to patients. I noted the lack of parenteral studies. We had heard from Dr. Chapin some of the limitations in the Arcadi study and others. We would love to have a study that had been done on intravenous exposure that we have done similar to some of the ones that we had seen from oral exposure. So we even have fewer studies to assess for parenteral routes of exposure. And renal effects -- we are seeing renal effects in some experimental animals following oral exposure. We are not really seeing that

following IV, but that could be to some extent because we haven't looked hard enough.

In the absence of parenteral data, we would like to develop some methods for route-to-route extrapolation of dose, notably a PBPK model, and I understand that there are some efforts underway to do that, and I think Dr. David will mention some of that work.

Again, we would like to get some more accurate exposure estimates based on current clinical practice, not what was done 10 or 20 years ago. We would like to pay particular attention to children or hypovolemic patients as potential sensitive subpopulations for the effects of DEHP. We heard that to some extent. We had also heard initially what some of the benefit effects of DEHP are on red blood cell survival. I think it is interesting -- and these studies have not been extensively talked about -- but we know that DEHP inhibits phospholipase A and some of the lipo-oxigenases to inhibit the production of prostaglandins and other metabolites that occur from the arachidonic pathway. At least for local effects, DEHP exerts an anti-inflammatory effect. The potential exists that DEHP leached from PVC could exert an anti-inflammatory effect as well, and I think that is an endpoint that merits

further consideration and research.

We also need to keep -- you know, we are very focused on DEHP. We need to keep in mind that DEHP can be converted to MEHP in stored plasma, and that risk assessments need to be conducted for MEHP as well as DEHP. So let me finish my talk there, and if we can hold questions until the question and answer period.

I would like to introduce our last speaker, and that is Dr. Raymond David. Dr. David is a toxicologist at the Eastman Kodak company. He has also been very involved in the CMA phthalate ester panel, and I think he will be describing some work that has been sponsored by that group.

DR. DAVID: Thank you for staying. This is -- I have one of those enviable positions in the program of having a talk just before lunch. I guess that is second only to the person who has to speak right after lunch when everyone is half asleep.

What I would like to do is talk to you a little bit about the ongoing research of the phthalate esters panel. Certainly you have heard that there are a number of studies available on DEHP. I think in my own personal library, I probably have more than 500 or 600 articles and reports on DEHP and its toxicity. And yet, with this kind of a substance that is very well studied,

there are still certain scientific questions or uncertainties that we are trying to address.

So what I would like to do is just go over some of those, particularly as they pertain to this forum. One of the things I want to talk about are simply the physical characteristics of DEHP. There is quite a bit of information in the literature about DEHP and what its water solubility is, lipid solubility, et cetera, and some of those values may not be accurate. I also want to identify some of the key toxicity issues and concerns. You have heard some of them expressed here already. And to show you what the panel -- what the producers of DEHP and other phthalate esters are doing to address those particular concerns.

First, let me talk about the physical/chemical characteristics. If you go to the open literature and you look at what the water solubility is for DEHP -- for example, you can find values that range from somewhere around 6 micrograms per liter up to over 300. It turns out that based on a computer program -- a computer analysis done by the EPA laboratory in Athens, Georgia, the value is actually more like 3 micrograms per liter, and in fact this number has been verified experimentally using a slow stir technique to evaluate solubility. So some of the values that list DEHP's water

solubility as 300 may not be completely accurate.

Also, the octanol/water partitioning coefficient indicates that this substance is probably something on the order of 8-fold more soluble in lipid than it is going to be in water. Vapor pressure is very low, so that at ambient temperatures, we would anticipate very small concentrations of DEHP to be present in the air.

What do these values mean in terms of the impact on exposure and toxicity. Well, first of all, you would not expect very high concentrations of DEHP to be present in saline bags, IV tubing that come in contact with water. Also, the partitioning is such that you would expect more DEHP to be present in the cell membrane than in cytosol, and in fact we have already seen some data to indicate that there is greater concentration in the cell membrane than there is in the cytosol. You would also expect very low concentrations in vapor. So if you are talking about a PVC tube used for respiratory therapy, you simply would not expect to find a great -- a very high concentration of DEHP to be present in the air, especially if it were humidified air.

So does that mean that there is no exposure to DEHP? Not at all. I am not trying to imply that. I am only trying to give you a healthy scientific

skepticism in evaluating some of the information with respect to concentrations found, and to make sure that as you view the information, you keep in mind that DEHP is one of the most common laboratory contaminants. In other words, it is used in a great many products that are found in the clinical laboratory.

Knowing that there is concentration, though -- exposure of patients -- let me turn to some of the scientific concerns that we have and that our research program is trying to address. I have put them into three general categories. One is what is the mechanism of reproductive and developmental toxicity? And I put after oral exposure because as you have heard, most of the information we have, and in fact most of the effects that we have observed, have been following oral administration and very little following parenteral administration.

So we want to try and identify what those mechanisms are for reproductive and developmental toxicity. Will that mechanism be applicable to intravenous administration? I think they will be, and I will show you how later on.

We also want to better understand what the mechanism is for hepatocellular carcinogenesis after oral administration. Certainly there is a great deal that we now know about hepatocellular carcinogenesis in rodents,

and whether or not that is applicable to humans than we did 20 years ago. But there are still some questions that, particularly as they pertain to DEHP, that would help resolve lingering questions in the minds of some people.

Also, what we want to do is look at the applicability of the rodent model to humans. There have always been questions about just how applicable is the rodent model to human exposure. In many cases, it appears that the rodent model is not the best model to evaluate human toxicity.

So let me first turn to reproductive and developmental toxicity. Knowing that there is reproductive and developmental toxicity a few years ago suggested, and as Dr. Chapin already suggested, there is some question about whether or not DEHP acts as an endocrine disrupter. So one focus of our research program has been to evaluate whether or not DEHP can act as an estrogen. We set up a program in which we tested DEHP and its primary metabolite, MEHP, along with a number of other phthalate esters in an in vitro system using five different assays. Looking at binding to the estrogen receptor as well as activation of the estrogen receptor in four different cell types -- MCF-7, which is breast cells, HeLa cells, which is uterine cancer, and

the yeast cell. In all of those studies, DEHP was found not to bind to the estrogen receptor, nor did it activate the estrogen receptor. So there were no consequences that we could see.

MEHP, if we test it in vitro, does bind to the estrogen receptor, but there is on activation. If we look at it in the cell system, even though there is binding to the receptor, it apparently is a very non-specific binding because we can not get any activation. We wanted to follow up those in vitro results indicating or suggesting at least that DEHP is not an estrogen with in vivo tests using two different assay systems. One is a uterotrophic assay, which measures the uterine weight increases in ovariectomized animals, and looking at vaginal cornification, sort of a mimic of the estrous cycle. We found in both cases that DEHP did not demonstrate an estrogenic response. And those were at dose levels of anywhere from 20 to 2000 mg/kg. That information actually supports the conclusion by Milligan, who also looked at a uterotrophic type of assay in mice and also found DEHP not to be active.

So it would appear that DEHP is not an estrogen. It is not acting as an estrogen. But there may -- we are now looking at whether or not DEHP can act as an androgen or anti-androgen. And we currently have

a program ongoing, again looking at DEHP and the mono-ester to see whether or not it will bind to the androgen receptor or whether it activates the androgen receptor in two different cell types. We want to see whether or not it can activate the androgen receptor and whether or not it can block the activity of testosterone in the androgen receptor assay.

The results from Earl Gray that Dr. Chapin referred to suggested that DEHP doesn't bind to the androgen receptor. We anticipate that once those in vitro studies are completed, we will then move on to an in vivo assay of androgenicity, just as we did with estrogenicity. The likely candidate for an in vivo model is the Hershberger assay, one that was recommended by the EDSTAC. We would like to see that particular assay validated first, or any in vivo assay that we use, we would like to see validated before we move forward. But it seems likely that that is one possibility for an in vivo assay.

The scientific rumor is that that study has already been done and in fact is negative for DEHP and other phthalates. If that is the case, that may not be much of a surprise if in fact the androgen receptor -- that DEHP doesn't bind to the androgen receptor.

So if there are non-endocrine mechanisms, we

want to make sure that we pursue those as well. One possibility or one avenue of research is to identify what the active metabolite is for developmental toxicity. Per Sjoberg showed back in the mid-1980's that he could identify the reproductive toxicant -- in other words, the metabolite of DEHP that produced the testicular effects that were observed. But no one to the best of our knowledge has ever identified the developmental toxicant. And so what we are doing is using a rat embryo culture assay. We are incubating nine-and-half-day embryos with serum from rats that have been exposed with very high doses of DEHP. Once we can characterize the effect on the embryo, we are then going to go back and incubate those rat embryos with serum from control animals, but where we will reintroduce different components that we isolate from the serum, different metabolites. And in doing that, we should be able to identify which of the metabolites gives us exactly the same characteristics at exactly the same kinds of concentrations that we would find in the whole serum.

We also are looking at whether or not metallothionein induction can limit the bioavailability of zinc. That is a theory that has been proposed for many years now that zinc being an essential element for the development of the fetus and metallothionein being a

very inducible protein in the liver, that one could induce metallothionein in maternal liver, sequester the zinc from the fetus and thereby inhibit the proper development of the fetus.

There are some data -- Peters suggested that that was in fact the case for animals treated with very high dose levels of DEHP. The results have been rather unsatisfying so far in terms of identifying that particular mechanism, especially when it comes to reproductive toxicity or the developing reproductive tract in rodents.

Another avenue that we are pursuing is just to better characterize the reproductive and developmental toxicity. Dr. Chapin already told you that we are involved separately in studies trying to develop better data on what the effects actually are for DEHP. We have a two-generation study currently ongoing. I think at this point we are in the second generation. Animals are being treated with dietary amounts of DEHP ranging from 100 to around 900 milligrams per kilogram. And we are using our state of the art or at least current guideline methods for evaluating the reproductive effects. So looking at anogenital distance in males, looking at preputial separation and vaginal patency and all of the other early landmark parameters that are associated now with

conducting a good two-generation study.

We also have included in this study design looking at the testes of the pups that were exposed in utero, but looking at them using electron microscopy, so that we do not miss any very subtle effects that might be present or might be overlooked using a light microscopy.

We want to better -- once we get a better understanding of the mechanism, we want to try and assess what the actual risk is to the human population for reproductive and developmental toxicity. And so we have a number of studies that are possible that we are considering to evaluate that risk, one of which is, again, back to the rat embryo culture study. If we can grow rat embryos in serum from primates, we can evaluate whether or not there are active metabolites in primate serum that would adversely effect the development of rat embryos. That is not an easy experiment to do -- I see Bob smiling. He knows. Because there are going to be limitations to how much primate serum you can add to the medium before the rat embryo simply stops growing. There are, of course, going to be some nutrients that are peculiar to the rat.

Another possibility is to identify what the active metabolite is in rats and see if we can find that

metabolite in serum from DEHP-treated primates. Further, what we want to do and in fact we are in the process of doing is conducting pharmacokinetic studies using pregnant primates and rats. The objective is to look at the tissue dosimetry to the fetus. We have selected marmosets as our primate. Every time I explain this to groups, I frequently get a question, why did we select the marmoset? Why not an African green monkey or a cynomologous or rhesus monkey? Actually there are, I think, some legitimate or valid reasons for selecting a marmoset. First of all, marmosets typically have two kits or two offspring per pregnancy. Quite honestly, that gives us twice as much opportunity to measure the dose to the fetus than it would from an ordinary primate or for a different primate. We already have some pharmacokinetic data for marmosets. And in fact we have some data indicating that marmoset seems to be resistant to at least some of the effects that we see in rodents.

The question of whether or not a marmoset is a good representation of a human is frequently asked, and that was reviewed by Llugenot and Cornu back in 1995, and their conclusion was that the metabolism in the marmoset is equivalent to the metabolism in a human. So this should be a good model.

The study set we are conducting incorporate single and repeated administration. Not only oral administration but intravenous administration. How long those exposures will continue is something we need to decide, because we want to try and capture these sensitive periods for all of the various endpoints. And short of dosing the animals gestation, I am not sure if we can incorporate all of them into a short period of time. But we certainly want to have repeated administration and repeated IV administration, which I think will help some of the issues in question for the FDA.

We want to look at the amount of metabolite in the placenta and in the fetus to determine whether there is transplacental transfer. It is quite possible that we could find a fair amount of DEHP or MEHP associated with the placenta itself, simply because there is a great deal of membrane there and an exchange. But we certainly want to determine the body burden for the fetus. We focused primarily on the fetal liver and testes. There have been questions about whether or not we should include the kidney as another target organ, and that is something that we can certainly consider.

The information that we gather from this will help us develop a pharmacokinetic model that we can

then use to assess risk in humans from human exposure short of conducting a developmental toxicity study in a primate.

Let me spend just a few moments talking about the studies that we have ongoing on the mechanism of carcinogenesis. Dr. Cunningham has provided a great deal of background information about what is currently known about the mechanism of carcinogenesis. I have just listed some bullet points here, and I only want to add one or two things. Certainly PPAR alpha is an important or essential part for liver carcinogenesis based on the Wyeth 14643 study. Humans and guinea pigs have fewer receptors. I point out guinea pigs, not that they are particularly close to humans, but they then give us another animal model that may be similar to humans that we can then use in experimentation. In fact, the guinea pigs are the ones that have been shown to have also an inactive response element.

One paper that just recently came out indicated that one could separate the peroxisome proliferation response from the hypolipidemic response, at least in rabbits. And I would be curious to find if that were also true for other species such as humans, which means you could clearly have therapeutic effects from peroxisome proliferators such as fibrase, but there

would be less of a concern with respect to carcinogenesis.

So the studies that we have planned using DEHP and MEHP is to look at peroxisome proliferation in human cells. Now, that is not really something new. That was demonstrated back in the mid-1980's by Cliff Elcom. But we also want to include evaluating cell proliferation and apoptosis, because those two have not been evaluated in the human liver in response to DEHP or MEHP. And we will compare that to the effects in guinea pigs, thinking that those two species may act similarly since they are both insensitive.

We will then look at the response element in human cells using acyl CoA oxidase as a marker. And even though this has been demonstrated with another peroxisome proliferator, we want to demonstrate it with DEHP to clarify any further issues about whether or not DEHP can act the same or differently from other peroxisome proliferators.

Given all the research that is ongoing, there are still certainly some uncertainties that remain. We have heard these issues brought up throughout. Questions remain about whether or not repeating the Arcadi study, knowing that they didn't measure the levels in the water and knowing that they didn't measure water

consumption and knowing that the water solubility is very low. If we repeated it, would that resolve the question about the biological effects that were observed? Quite honestly, there have been a couple of studies in the past few years where dramatic effects have been observed in animals receiving very low concentrations in water. And when we try and go back and attempt to replicate those results using more animals and using better analytical controls and even conducting the studies according to good laboratory practice regulations, you don't get the same kind of biological effect. And yet, the studies are still in the literature. So I don't know whether repeating this will actually clear up whatever uncertainties exist.

Or if we are talking about carcinogenesis and we find that in the human liver samples that are already tested there are decreased levels of PBAR alpha or the response element is in fact inactive, will we have to go back and test a great number of human liver samples to evaluate whether or not there are subpopulations that exist that may be more sensitive than the samples that we have already seen.

Ron Brown suggested that doing a primate study was a key issue or concern for the FDA. I guess we have to wrestle with should we try to repeat a primate

study using what would be current techniques of blood or plasma infusion, and would that provide us information if we already have data from other animal studies or from humans that fail to show any kind of hepatotoxicity. Or should we conduct an intravenous developmental toxicity study if we can identify that the developmental metabolite, the active metabolite, is not present in primate serum or in human blood? Or that the amount that actually reaches the fetus is very small? Or that the exposure, based on the procedure used, will provide very little or no metabolite.

So let me just summarize quickly. I think we can actually break for lunch pretty soon. The physical/chemical properties are such that the environmental exposure certainly is much lower than many people believe, and that it is quite possible that some of the environmental exposures, even in the emergency room or in a clinical setting, may be lower than some people expect. We know that DEHP is not estrogenic, and we are in the process of evaluating whether it is androgenic or anti-androgenic in the classic sense of the word.

We certainly want to characterize the human risk for reproductive and developmental toxicity, and there are a number of ways that we are using to go about

to determine that. We are also determining the mechanism of carcinogenicity to determine whether or not there is potential for carcinogenesis in humans.

I think although the data suggest that there is no risk or little risk, if any, I think from exposure, we realize that there are certainly unanswered questions and we are trying to be very responsible in responding to these questions and to determine what the effects really are. Thank you.

MR. BROWN: Thank you, Dr. David. I would like to invite the speakers from this session to join Dr. David up at the table. And I thank all of the participants for their patience this morning. I know we are running late. But I would like to provide an opportunity for about ten minutes of a question and answer session. I think that will get us to lunch right around 12:30. Since I understand there is a cafeteria downstairs and upstairs I am told -- so there are several options for the fine NIH cuisine -- that perhaps we can reconvene on schedule and then we would be all set. So let me ask if there are any questions for the speakers in this second session today.

PARTICIPANT: Herb Cullis, American Fluoroseal Corporation. I would like to ask the panel if they have any comment on the toxicity of DEHP for human

leukocytes for transfusion. That is my question. My preface is this. In 1983, Stevenson reported that monocytes could not recognize antigens if they had been stored in DEHP plasticized vinyl bags, and he went on to develop a teflon bag for the purpose of storing and generating monocytes for transfusion. Later, the Lacsell Therapies here found that lymphocytes could not replicate when grown or when attempted to be grown in DEHP plasticized vinyl bags, and eventually Baxter developed the life cell bag, which had I think about -- Joy will correct me -- about 8 percent of the DEHP that the previous bags had and that permitted some growth of lymphocytes in bags. That elimination of all DEHP from those bags provides about a 30-fold improvement in replication of lymphocytes. Later Daisy reported that CD34 positive cells will neither replicate nor differentiate when stored in DEHP plasticized vinyl bags and went on to develop another method for culture. Whereas I think Dr. Ness and Dr. Snyder discussed the red cells and platelets, these are fully differentiated cells, and the effect of DEHP may not be seen. It would seem to me that the model -- or actually not the model but the real thing is the human lymphocyte. The information has been around for at least 15 years. And my question again to the panel is do you have evidence or

comment on the mechanism of toxicity of DEHP on the human leukocyte?

DR. KARLE: I don't know of any. But as a practicing neonatologist, I don't desire to have white cells transfused to my babies when I am transfusing packed cells. The reactions and issues of infection are more related to white cells. So it is not something I am concerned about in my patient population at this point in time.

PARTICIPANT: I'm John Butala. I am a toxicology consultant working for Aristech. I have a question for Dr. Chapin. Can you hear me, Bob? Can you hear me now? The question for Dr. Chapin is that you made the point in your presentation that with regard to reproductive toxicity and DEHP, it is important to look at reproductive function and to look at that function in animals that were exposed prenatally and then followed, of course, post-natally. And then you showed us two studies, one by Jim Lamb that looked at males and females and had a relatively high NOAEL for this, and then one by Cottie that had a low one. And then you kind of tantalized us a bit, I think, and you told us about Dr. David's study and your own study, the multigeneration repro type studies. And then my question to you then is do you anticipate data coming from one or both of these

ongoing studies that will somehow help interpret the two studies that you did tell us about, and would you care to speculate on how you might integrate these data?

DR. CHAPIN: The studies that are ongoing are measuring -- are unique from the studies that I described this morning in that the new studies will incorporate both functional assessments, which is to say they will breed the second generation -- so functional assessments along with the structural and developmental milestone measures that are currently believed to be sensitive for finding antiandrogenic activities or estrogenic activities of compounds. So the Lamb study did not evaluate preputial separation or anogenital distance or any of those measures of androgenic status in animals, and did not evaluate the reproductive function of the second generation. Arcadi did not evaluate reproductive function. He looked a little bit at structure, but not at all of the endpoints that we are currently concerned about.

The study that Ray described and the study that we have got ongoing under our auspices will do both of those same things. Those will help put these other -- the Arcadi and the Lamb studies -- into some context. But in truth because they are so much more inclusive in terms of endpoints, I am not going to worry yet about our

ability to or how we will worry about sort of folding all these data together until I see the data.

PARTICIPANT: Daland Juberg with the International Center for Toxicology and Medicine. A very similar question to either or both Dr. Chapin and Dr. David. You both mentioned you have ongoing two generation reproduction studies. Given what we know, would a next logical step depending on the results of those be to evaluate the same study using the IV route of exposure? Would that be practical or relevant?

DR. CHAPIN: Ray, I think I will let you do that study.

DR. DAVID: Oh, no, Bob.

DR. CHAPIN: My sense, Dal, is that the best thing to do would be to find the key, most sensitive effect in a multi-gen oral study and then to target the appropriate exposure time using the IV group. And hopefully that is going to be either -- that will probably be some developmental sort of exposure window. So maybe between the CMA panel and the NTP, we can come up with some design that we are all happy with and see if we can sponsor something like that together. Who knows. Anything is possible. But something like that would be an interesting thing. I was wondering if there was a way that we could -- if we are missing a boat here and maybe

lymphocyte or white cell responses ought to be factored into these things somehow. Maybe there is a way that we ought to be adding that under our studies as well so that we can compare those kind of endpoints along with what we currently think are the most sensitive reproductive developmental points. Maybe we ought to piggyback some of those things together.

DR. DAVID: I think just to continue what Bob said, I have concerns, I have to tell you, about the experimental design for repeated administration, say, to rodents. I know that there are very good techniques for in-dwelling catheters and perfusion over time. And certainly I am aware that there are laboratories that are very good at doing that. I guess my approach might be first to look at whether or not you could use other techniques such as say pharmacokinetics -- you know, looking at pharmacokinetic modeling and metabolism and identifying metabolites as one first step before going to the step of actually doing the study. So maybe from that perspective, Bob and I differ in our approaches a little bit.

Probably it is going to be necessary to have some kind of an evaluation of those endpoints in a study that encompasses what we agree are the sensitive time points or time frame during gestation. What the model

species is and exactly how we do them I think is still something we would need to talk about.

MR. BROWN: Since it is 12:30, maybe we can just have one final question.

DR. SNYDER: Yes. This is sort of in response to what Herb Cullis had commented on. As a blood bank director who --

MR. BROWN: Could I just ask you to state your name and affiliation?

DR. SNYDER: Oh, I am sorry. Ed Snyder from Yale University. As someone who does a fair amount of activity with the oncology program, the comments that Herb made about white cells and the effect of plasticizers may be true, but it should also be remembered that over the years the collection of CD34 positive cells for transplantation in machines developed -- Amicus, Kobe, Hemonetis and a variety of other companies, Procscenius -- have resulted in engraftment in 8 to 9 days for granulocytes, and for platelets 10 to 14 days routinely. Donor lymphocyte infusions, CD34 positive selection with T cell negative selection and tumor selection using devices that have tubing that contain varying amounts of DEHP and a whole variety of other plasticizers all belie the fact that there is an acute toxic effect of these materials. That doesn't mean

they shouldn't be looked at, but clearly I am not aware of any studies that have shown any toxic effects from these materials. So I don't want -- I think it just should be stated that clinically it doesn't appear that there is a problem. But nevertheless, we may be able to do better or find that removing some of these plasticizers may be of value. But right now clinically, they seem to work quite well, even though they are not end state cells as you appropriately pointed out.

MR. BROWN: Okay, thank you. I would like to thank the panel and remind you that we would like to convene the third session promptly at 1:30. Thank you.

(Whereupon, at 12:32 p.m., the workshop was adjourned for lunch to reconvene at 1:35 p.m.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

1:35 p.m.

MR. HWANGBO: Now we are going to have the third session, the alternative to the current blood bag materials. Today, we have three manufacturers -- representatives from three major blood bag manufacturing companies. They are manufacturing various blood bag systems with different plastic formulations and with a different plasticizer concentration.

As you know, currently we can store red blood cells for 21 days or 35 days or as long as for 42 days in the refrigerator depending on their plastic film or depending on the anticoagulant solutions. We can store platelets at room temperature up to five days.

Our first speaker is Dr. Joy Anderson from Baxter Healthcare Corporation. She is a Senior Director, Medical and Scientific Affairs in the Whole Blood Technology Group of the Fenwal Division. Her talk will be interesting in that she will discuss manufacturing requirements, which we cannot ignore, and the blood bank perspectives as well as the viewpoint of hospitals. Dr. Anderson?

DR. ANDERSON: Good afternoon. Could I have the first slide, please? Over 30 years ago, plastics revolutionized transfusion therapy. The replacement of

glass bottles by plastic containers allowed whole blood to be separated into red cells, platelets and plasma in a sterile closed system disposable. This meant that patients could receive optimal transfusion therapy by receiving the specific blood components they needed rather than whole blood. Patients who were anemic and needed improved oxygen delivery could receive red cells, while patients who were in danger of bleeding could receive platelets. These improvements in patient care resulted from the use of PVC-based plastic materials.

DEHP plasticized medical products are widely used. An estimated 5 to 7 billion patient days of acute exposure and 1 to 2 billion days of chronic exposure have occurred without report of significant adverse effects. There is no scientific evidence that DEHP exposure from medical products is a human health risk. Animal studies on DEHP cannot be directly extrapolated to humans receiving blood components. The human exposure levels to this plasticizer during transfusion are well below rodent toxicity thresholds. The rodent metabolism of DEHP is different from humans, and key rodent mechanisms such as peroxisome proliferation are different or absent in humans.

DEHP does migrate at very low levels in aqueous solutions. In lipid-containing solutions, such

as red cells and plasma, more plasticizer migrates. It is important to note that substances leach from all materials that contact or store solutions. This includes glass bottles, ceramics and both PVC and non-PVC materials. Glass bottles leach metals, salts and silicates. Ceramics leach some metals and organic materials. Therefore, it is important to look at the whole spectrum of material properties when choosing a material for a specific application.

In the case of red cells, a surprising benefit of DEHP was noted. The presence of DEHP resulted in significantly reduced hemolysis during red cell storage. This slide illustrates the protective effect of DEHP on red cells. The plasma hemoglobin level in red cells stored in a non-PVC container was 540 mg per deciliter, nearly twice as high as when red cells were stored in a PVC container with DEHP plasticizer. Thus, DEHP has been shown to improve the quality of transfused red cells by reducing hemolysis. This is one example of the unique requirements for the optimum storage of blood components.

The special challenges involved in developing blood container materials will be discussed from three perspectives; the manufacturing requirements, the blood center perspective, and the viewpoint of the

hospital. Any materials for the storage of blood have to meet the unique requirements of each of these environments.

From a manufacturing perspective, blood containers must be suitable for high volume production. World-wide, we estimate that over 50 million plastic blood container systems are manufactured each year. These plastics must have a number of other characteristics in order to be suitable for use. They must bond satisfactorily with a variety of other materials ranging from other plastic formulations to materials as diverse as the needle.

Blood container ports, which allow components to be transfused to patients, present a special manufacturing challenge. The ports must include an effective microbial barrier and also bond adequately to the plastic sheeting. The materials must be compatible with a variety of solutions, including the anticoagulant, storage solutions and the blood components themselves. They must be capable of withstanding high temperatures for a prolonged period of time when steam sterilization is used. In addition, the plastic material must be able to be manufactured into a variety of product configurations to meet varying customer requirements.

From the blood center perspective, there is

a requirement for low cost, sterile, single-use disposables. A variety of product configurations must be available to meet the need for collection, processing and storage of a range of blood products. For example, when the blood center produces red cells and plasma from whole blood donations, a double blood pack configuration would be used. And when the center produces red cells, platelets and plasma, a triple blood pack configuration would be required.

The materials used in blood containers must have a long shelf life so that the blood center eliminates the costs associated with unused, expired products. The materials used in blood packs must be kink resistant, so that the blood flows freely during collection and during component preparation.

Multiple centrifugations are required to separate whole blood into red cells, platelets and plasma, and blood containers must have the strength to withstand this high G force without leaking. The blood container materials must support the satisfactory storage of blood components under a wide variety of temperature conditions. Red cells are stored at refrigerated temperatures, platelets at room temperature, and plasma is frozen. In addition, the materials must provide adequate dating for each component. This allows the

blood center to efficiently manage the inventory of red cells and platelets and to always have blood components available when patients require them.

Transfusion therapy practices determine many of the hospital requirements for blood containers. Before transfusion of red cells into patients, the blood must be crossmatched to make sure that the blood transfusion will be compatible. The use of plastic tubing that can be made into segments allows these samples to remain attached to the red cell unit so that there is less chance for error. Flexible containers allow the maximum amount of each blood component to be delivered to the patient. Optical clarity allows a visual quality control check to be performed before the transfusion is started. Self-collapsing walls eliminate the need for the introduction of sterile air.

In addition these patient-related factors, there are additional requirements that have to do with blood product storage and administration. These include the strength to withstand shipment from the blood center, usage under pressure without leaking, and the capability for further aseptic processing using sterile connection equipment. The containers must also maintain their integrity during sudden, extreme shifts in temperature, such as moving fresh, frozen plasma from a minus-20

degree centigrade freezer into a 37 degree centigrade water bath for thawing.

PVC is one of the few materials that can consistently meet this diverse array of requirements. However, DEHP plasticized PVC is not optimal for the storage of all blood components. For example, other plastics have been developed for the storage of platelets because they meet the unique requirements of these cells much better.

Alternatives to DEHP plasticized PVC were developed for platelets, not because of a concern about safety but because these materials provided superior platelet storage. The materials used in platelet storage containers must allow for good exchange of oxygen and carbon dioxide in order to maximize the shelf life and viability of platelets. DEHP plasticized PVC is not as permeable as other materials, resulting in a platelet product that can only be stored for three days. PL2209, PL732, PL2410 and PL3014 plastics all provide superior platelet storage, and these are the containers requested by our customers. These materials provide a choice between a non-DEHP plasticized PVC and a polyolefin container, and all provide five-day platelet dating.

Baxter has ongoing efforts in materials development. We currently have non-DEHP alternatives

available for the storage of all blood components. This slide depicts the alternative materials currently available from Fenwal for the storage of blood components. All of these materials are non-DEHP. Some of them are also non-PVC. The materials vary in their ability to withstand autoclaving, in their optical clarity, and in their ability to be sealed using radio frequency technology. RF sealing is an important consideration in the manufacturing process. Optical clarity is a consideration from the user's perspective. Only those materials that can be autoclaved are suitable for use in blood packs.

An important thing to keep in mind is the amount of time, effort and money that goes into the development of biomedical materials such as those listed here. A manufacturer can't just decide to order a new material today and use it tomorrow or next year or even three years from now.

This slide illustrates the typical timeline for development of a new material, from the idea phase through implementation and manufacturing scale-up. The development process is complex and highly disciplined, typically requiring five to six years. The medical products industry is highly regulated and there are specific design control, regulatory and good

manufacturing practices that must be adhered to.

Baxter has invested approximately $200 million toward the development of alternative materials for a variety of applications. Of the research programs that have been initiated, approximately 50 percent have shown sufficient promise to undergo clinical testing and regulatory submission.

PL2209 plastic was developed as a single plastic that could store all blood components. The material is a citrate plasticized PVC, so it does not contain DEHP plasticizer. The material meets the blood center and hospital requirements for strength, optical clarity and flexibility. PL2209 plastic was approved by the FDA in 1991 for the storage of all blood components. It provides the maximum component dating of 42 days for red cells in additive solution, 5 days for platelets, and one year for fresh frozen plasma.

As part of the development process for PL2209 plastic, we performed non-clinical pharmacology and toxicology studies. The evaluations performed using the final plastic formulation are indicated by the letter P. Studies done using a plastic extract are indicated by the letters PE, and those studies using the plasticizer, BTHC, are indicated with the letter C. This testing included red cell and platelet storage studies, and acute

toxicity studies in rat, dog, mouse, rabbit and tissue culture models. We also performed subchronic toxicity studies in rat, neonatal rat and dog models. Peroxisome proliferation was studied in a rat model. Dermal toxicity, dermal irritation and ocular irritation were studied in rabbits. Fertility and teratology studies were performed in a rat model. Mutagenicity was evaluated. Pharmacokinetic studies were also performed including distribution, metabolism and excretion. There was no evidence of peroxisome proliferation or mutagenicity in any of these studies. Based on these results and according to well-accepted toxicology standards, carcinogenicity tests were not performed.

After the non-clinical pharmacology and toxicology testing, extensive clinical testing was per formed using PL2209 plastic. The clinical evaluation included in vitro and radio-labeled studies of CPD whole blood, CPD packed cells, and red cells in additive solution. Red cell antigen preservation was evaluated during storage in PL2209 plastic containers. Red cells that were collected in PL2209 plastic were also studied following freezing, thawing and deglycerolization. The studies conducted on platelets stored in PL2209 plastic included in vitro and radio-labeled studies as well as clinical transfusion studies in thrombocytopenic

patients. One year storage studies were performed on plasma with evaluation of coagulation factor and cryoprecipitate stability.

The development of this material required an investment by Baxter of more than $35 million. After market introduction, customers preferred to use blood packs manufactured from other approved materials. Because of the higher costs involved in producing PL2209 blood packs, this product costs 10 to 15 percent more. Customers did not see a need to spend more money for a product that was comparable to the one they were using. The blood pack configuration that our customers preferred and continue to prefer contains DEHP plasticizer in the PL146 containers used for red cell and plasma storage. PL732 plastic, which is a polyolefin material, is most often used for the platelet storage container.

This detail on the product development process and our specific experience with PL2209 plastic has been discussed to illustrate that the development of new materials is a complex, time-consuming, and costly process which is not always successful.

Fenwal has been a leader in the transfusion medicine industry. We pioneered the development of the plastics that have made today's component therapy possible. Fenwal has invested heavily in the development

of alternative materials. We have a variety of non-DEHP plasticized materials available for blood component storage. The specific product configurations we manufacture are determined by customer preference. Citrate plasticized blood packs were not accepted by customers although they met all the requirements for safety and efficacy, customer usage, and blood component storage. We believe that the array of materials currently available provides for optimum storage of blood components in a safe and efficacious manner. Thank you.

MR. HWANGBO: Thank you very much, Dr. Anderson. Our next speaker is Mr. Raleigh Carmen of Medsep Corporation, formerly known as Cutter Biologicals, a division of Pall Medical. He is Senior Vice President of Research and Development. As you remember, his paper was mentioned by our previous speaker and now you are going to see him in person.

MR. CARMEN: Ms Hwangbo, ladies and gentlemen, good afternoon. There is quite a bit of nostalgia at this meeting and I will try not to add to it too much. I hope you will bear with me.

Plastic equipment for processing blood and blood components was introduced by Carl Walter in the late 1940's. The material used in this equipment was known in the trade as soft vinyl, and this is really

polyvinyl chloride or PVC polymer blended with a chemical called a plasticizer to make it soft and flexible. Plasticizer, I believe, comes from the German weitmacher, which means soft maker.

It was in the mid-1960's when John Ottian and others first drew attention to the potential hazards of the use of plastics in medicine, and the specific issue relative to the extraction of plasticizer and specifically to DEHP plasticizer by stored blood components was first raised in 1970. Since that time, enormous amounts of time and money have been expended in the search for alternate materials. But after 30 years of looking, plasticized PVC remains the material of choice for blood bag systems today.

The reasons for this, Joy eluded to, is that the procedures used in the preparation of blood components together with the processes used to manufacture multiple blood bag systems impose a really unique set of requirements that a plastic must have to make a modern blood bag system.

I am not going to spend much time, because I think Joy did a fine job in just going over the requirements that a blood bag plastic must have. I do want to mention one thing relative to one point -- can be licensed -- that seems a bit trite. But there was one

example that I can well remember, a material referred to as thermoplastic polyurethene elastomer was studied by a number of companies. It really possessed almost -- it possessed all the properties required of a functional multiple blood bag system and it had no plasticizer. I am not sure it would have met this requirement of relatively low cost. But in any event, an enormous amount of time and money was spent on this, and this had to be stopped because of the potential for extraction, I believe in nanogram quantities, of methylene dianiline. So it is examples like this that show you the difficulty of this endeavor.

I can also attest from a personal standpoint of the difficulty of this. When Cutter was acquired by a German firm, Bayer, who have enormous expertise in polymers and practice. And once we were part of their family, they said just give us all of your materials problems, and we will take care of it. Of course our main problem at that time -- this was in the early 1970's -- was to find an alternate to soft vinyl. They said in the typical Germanic way, no problem, and began to work on it. Their approach was to use a modification of polycarbonate chemistry. Polycarbonate, you are probably aware of, is a very hard, strong, tough plastic, but it is possible to modify the polymer chemistry and make it

a flexible material. So this was the approach that they took. After quite a number of man years and a lot of Deutschmarks, they finally had to abandon this and frankly stop the project.

So while soft vinyl replacement is extremely difficult if not impossible, there are alternates to the extractable plasticizers such as DEHP. Free benzene ring buffs -- this shows the structure of DEHP and a potential alternate, which is -- the acronym is TOTM. You can see that the structure of these two plasticizers is quite similar. DEHP is an ester of a dicarboxylic acid or thalic acid, and TOTM is an ester of a tricarboxylic acid or TOTM. The alcohol moiety is the same in both cases, 2-ethylhexanol.

Now despite the similarity in structures, these plasticizers behave quite differently as regards the propensity to leave the plastic matrix and enter into contained solutions of particularly fatty media like blood and blood components. This is a comparison of the relative extraction rates of TOTM and DEHP from stored blood components. You can see there are two orders of magnitude or more difference. In the case of whole blood, a 10-unit transfusion would result in administration of about 400 mg of DEHP versus 1 mg of TOTM. The ratio is similar for platelet products. And

even at 7 days, assuming a 10-pack of platelets, the patient would be receiving about 210 mg of DEHP per dose versus 2 for TOTM.

Quite a bit of toxicology was done years ago on TOTM plasticizer. This is just a partial list of the studies that were done. The only adverse effect noted in all of these studies was in dogs and rats administered an extremely high dose given the extraction resistance of 42 mg per kg per day over a three week period. The no-effect dose was 14 mg per kg. A patient transfused with a pool of 10 platelet concentrates would receive about 0.03 mg per kg or about one-five-hundredth of the no-effect dose, so an extremely large safety factor.

At the time this work was completed, in view of the resistance to extraction and the safety, it was our plan to use this plastic, PVC with TOTM plasticizer, which we trademarked as CLX. The intent was to use this for the entire blood bag system so that all the bags, the tubing, and all the fitments and molded components in the fluid path would be of CLX plastic. This plan, unfortunately, was thwarted by the observation that you've heard about several times today that DEHP, by virtue of its migrating into the red cell component -- in fact, any plasticizer that migrates will probably do this. This has a salubrious effect on the red cell

membrane.

This has already been eluded to. In the absence of a -- in a non-extracting container such as CLX or PL732, there is increased hemolysis, quite a decrease in the morphology scores, and an increased osmotic fragility. And this effects noted in vitro were confirmed to be a problem in vivo, as you have heard about before. Some of the earlier work was done by Byron Myhre. As you can see in the case of 21-day storage, there is absolutely no issue at all. So you can store red cells in CLX for 21 days with no problem. However, when you go beyond that up to 35 days, as you can see there is quite a drop off in 24-hour survival, and Jim AuBuchon has already given these data earlier this morning.

So because of these findings, we had CLX licensed only for the storage of platelets and plasma, and red cell storage in the satellite bags was limited to 21 days. That is probably a very rare thing done in practice anyway. However, we continued to try to find ways of extending red cell storage in non-extracting containers such as CLX. We learned quite some time ago that by the maneuver of removing leukocytes from the product prior to storage, we would reverse some of the effects that were noted. Particularly the increased

hemolysis was pretty much completely reversed by the maneuver of pre-storage leukocytes reduction. The morphology scores, while not completely corrected were somewhat corrected as well as the increased osmotic fragility was partially reversed by removing the leukocytes from the product prior to storage in an unextracting container.

We then looked at whether this maneuver would give us satisfactory in vivo performance with storage in a conventional preservative. This is a busy slide. All I want to point out is the studies done by Andrew Heaton of red cells stored in AS3 preservative where the leukocytes were removed prior to storage. The results, although not good enough for licensure, were encouraging, particularly in the case of the single label, although the double label method everyone considers more rigorous.

What these data suggested to us is that we could probably bring this about if we would improve the preservation media in conjunction with prestorage leukocytes reduction. We selected for study the approach put forth by Harry Merriman and his colleagues. The principle of their preservation media was to use a hypotonic medium to induce osmotic swelling and an increase in cell surface tension, thereby forestalling

the shape change usually associated with stored red cells. Another maneuver was to have a medium that was low in chloride, and this was done to increase the intracellular pH via a chloride shift and also the extracellular pH was increased over standard preservation media.

The solution that we settled on is designated AS6. That doesn't mean that it is licensed, but it is designated AS6. As you can see, it contains no new chemicals. These are all used in practice in one formulation or another throughout the world -- glucose adenine, mannitol, phosphate and citrate. The pH is alkaline, 8.3, and it is hypotonic with an osmolality of 196 millisomoles per liter.

After getting encouraging in vitro results, we went and did red cell survival studies, and these are summarized in this final slide. This is the test showing that the red cells stored in AS6 preservative and stored in a non-extracting container do meet standard for red cell -- this is 42-day, by the way -- 42-day storage -- do meet standard for storage, which is 75 percent. There is still a slightly less survival compared to a DEHP control. These are just barely statistically significant, but I don't see that they could be called clinically significant.

So our plan is to attempt to have this system licensed and introduce it to the marketplace. The acceptance, I would not want to predict. There will be some added cost to this system because the solution, as you probably noted, has a high pH and therefore cannot be autoclaved as a single component. It will require two components. This is something that is done in Europe actually now. But making blood bags in this way does add to the cost. Thank you for your attention.

MR. HWANGBO: Thank you very much, Dr. Carmen. Now we would like to invite our last speaker, Mr. Jeff Miripol of Terumo Corporation. He is the head of the business unit. His headquarters is in Somerset, New Jersey. His talk is going to be the reality of blood collection and storage. He is saying why we are where we are.

DR. MIRIPOL: I think I'd rather move around a little bit. Can you all hear me all right? Again, thanks very much for allowing me to come and speak with you today. I wanted to give a little bit of a different view of blood storage and the effect of plasticizers.

What I am going to do in my talk today is give you a very brief history and then a review of the benefits of plasticized vinyl blood containers, many of which you have already heard a number of times and were

very well summarized by both Joy and by Raleigh and others. A little review of doses and some of the technical usefulness and utility of plasticized vinyl materials, and then finally a little argumentative polemic, ideals versus achievables. Maybe I won't be too argumentative.

Of course, prior to plastics, blood was collected and stored in glass vacuum bottles. Sterilization issues, breakage, you couldn't do component therapy, glass hemolyzed red cells actually fair actively, there is no gas exchange, it is an open airway system for both collection and for the transfusion of blood. As Joy indicated before, it is also truly not a non-leachable material.

As was I think mentioned in earlier speakers, Carl Walters from Massachusetts developed the DHP plasticized vinyl blood bag, which was really his, a surgeon's, response to the glass bottle situation. He was concerned about air entry into the blood product. He was concerned about sepsis and so forth. I have to give credit to Dave Bellamy and the group at Fenwal in the late 1950's and 1960's that developed actually the vinyl formulations that could be manufactured in a routine manner.

Again, to reiterate what has been mentioned

before, from a manufacturing standpoint, vinyls and plasticized vinyls in particular have a lot of advantages. Very well understood manufacturing processes. They lend themselves to high degrees of automation, a high degree of cost reduction and control of the materials. Relative ease of sterilizing the product after the manufacturing process, and this should not be ignored because it is very expensive to actually try to sterilize solutions by sterile filter techniques. These materials obviously have a low shipping weight, very little breakage, and there is a very high level of both manufacturing and shipping safety. And the end result is that you have a very low -- or relatively low, I don't want to say very low -- a relatively low cost to the final user for each bag.

At the blood center, once again to review and mention many of the same things that Joy did, you've got a product which is very easy to ship and to store. It is very flexible, which allows you to do a lot of things with it. It is a completely closed system and it is an expanding blood bag with no airway, of course. And you can make, as was mentioned many times, multiple components -- red cells, platelets, plasma, cryo, et cetera.

Also, you can spin the containers in the

centrifuge. You can store bags over a wide range of conditions. Here I am speaking only of DEHP plasticized vinyls from minus-40 to 22 degrees for platelet storage. The plastic materials allow CO_2 to go through and for oxygen to go through. And as has been mentioned, DEHP greatly reduces the red cell storage lesion. And also there is a very high level of worker safety. You are not working with glass materials that can fracture, et cetera.

At the hospital transfusion site, you have got a product -- again, as has been mentioned -- that can handle a wide range of temperatures. You can use it in the water bath. You can use it in the freezer. It transports easily. Again, no airway when you transfuse. You can infuse platelets, red cells, et cetera, under high pressure conditions in the ER, et cetera. And you can also, because the system has plastic tubing, et cetera, you can add on in a sterile fashion filters and other bags and so forth.

Finally, and this is a point that should not be ignored from a cost standpoint, these containers can be thrown away very cheaply basically. They are highly safe. They can be incinerated. And again, the factor of low weight is, I think, very important.

The patient gets a lot of benefits from

this. They have a closed system for the collection, storage and processing of blood. The patient has much less of a risk of getting sepsis. Obviously, they are able to get component therapy. They get a blood component that is a better blood component. And then they also have a high cost benefit here. The bag cost is typically not much more than 5 percent of the total patient's billed cost for the blood. You are talking about a product which is in many respects from a technology standpoint quite complex, but also quite inexpensive.

So we as a society, what do we get? We basically are able to get products that allow us to give specific blood components for specific patient needs. If we didn't have component therapy or if red cell storage was reduced to 21 days or if platelet storage was reduced to 24 hours or if we were using glass bottles, et cetera, our estimates are that there would be at least a 30 to 40 percent increase in blood shortages, and that there would be probably a four time increase in bacterial sepsis. This is very conservative. The other issue is that it would be very difficult to serve needs overseas and of course during earthquakes, floods, et cetera.

So going back to a review, again, of DEHP as a plasticizer for these vinyls. Again, as Joy indicated,

it is virtually the most widely studied plasticizer. It appears to have little if any effect on humans. Chronic exposures -- and again, there is a wide range of studies -- but in appropriately handled materials, i.e., materials where DEHP has been extracted from blood bag materials, not added need et cetera, the chronic exposures may be up to 6 mg per day. Patients undergoing dialysis, again a very chronic situation, exhibit blood levels of up to 14 micrograms per ml post-treatment, and of course they do exhibit some MEHP. But, again, the toxic effects of this are virtually not seen. Maybe they are not well understood, but I would contend with actually almost 40 to 45 years of use of DEHP plasticized vinyls, one would have expected at this point that we would be seeing some sort of a great problem in this area, and we just don't see it.

Once again, plasma phoresed donors or donors undergoing cell phoresis do not exhibit any levels of DEHP, and this is from work of the early 1980's. Patients receiving cryo -- they may receive as much as 5 mg of DEHP per week. Again, they don't exhibit any DEHP or MEHP in their blood. Again, that is from the same workers, Tucchi and their group in 1982. True, the doses of DEHP during an acute transfusion situation might reach as much as 30 to 100 mg per each transfusion under very

extreme conditions, and blood products may provide up to 70 micrograms per ml per infusion. But again, no known side effects have been observed.

Joy's numbers are actually greater than this and my numbers are probably five years to eight years out of date, but what I am showing here is in terms of exposure to DEHP. That number is certainly over probably one billion with blood therapy and IV use. Chronic exposure in excess of 5 million patients undergoing dialysis for multiple years. And once again, we are not seeing any sort of problem effects with these patients due to DEHP or due to MEHP.

Of course as we've all been discussing, we would like to find different materials. Baxter has done a very nice job looking at other materials. Terumo has also looked at both other materials and ways to reduce the extraction of DEHP. We have developed some formulation changes that result in a little bit reduced DEHP into whole blood. We have looked at longer chain phthalates. We have looked at vinyl acetates, olefinates, et cetera. In the last 7 years, we have spent over $15 million looking at various materials.

Our results, quite frankly, are not all that fantastic. We are able to reduce extraction levels so that we can meet the Pharmacopoeia in Japan, which is a

little stricter in terms of total plastic by-products. But again, we still need to have DEHP for red cell protection. The other materials that we have looked at and the other formulations cost as much as one-third to almost three times as much, and they suffer from a lot of other limitations.

Before I go on to the advantages, the limitations, as were mentioned, in terms of these other materials have to do with the fact that DEHP plasticized vinyls -- not only do they afford red cell storage improvements and protection, but you also have a system which is easy to manufacture, is good at low temperatures, and actually you can make bags that can store platelets, plasma, et cetera, from the same basic sorts of plastics. So the other advantages, of course -- and we have discussed this now a number of times during the afternoon -- we get greatly reduced red cell lysis. We have a material that breathes well, has good low temperature characteristics. It is not the ideal very low -- it won't work very well at temperatures down to minus-70, but it will work in a broad range of temperatures. Again, it is a material that is flexible, easily manufactured, low cost, high benefit. And again, there is really a lot of years of use with no apparent untoward effects.

So is there a single ideal material for blood collection, storage and processing? Again, as Baxter has indicated, there may not be one single plastic that is ideal under all conditions. We have, I think, probably areas we can agree on in terms of what we would like to have a plastic do. We would like to be able to store red cells for at least 42 days. Again, as Raleigh indicated, they are trying to do it by use of a hypotonic solution, which could possibly work but has some other problems as well as possibly higher expense. We, I think, are agreed we want to store platelets for at least five days and possibly more. We want plasma storage for multiple years. We want a material that is clear, collapsible, airtight, has got ports, tubings, you can label, et cetera, and it can be sterilized after manufacturing and is low cost.

If we are looking for a material that is totally benign, what does that actually mean? Well, again, other materials do not allow red cell storage to 42 days outside of the vinyl materials with certain plasticizers. Other materials don't handle as well in the laboratory or they also have problems at the bedside. They don't actually collapse properly. They may cause other problems in the blood center or in the hospital. For instance, transport problems. You may not be able to

label them properly, and so forth. And then again, they may cost much more. Again, some plastics may be too permeable and others not adequate.

So my concern here is consequences that we don't intend to actually or expect to see -- unintended consequences. For instance, in the recent past, one manufacturer was able to change the plasticizer, but that did lead to some customer problems, including loss of donor labels. It was a higher cost material. We also looked at changes in Europe to a non-vinyl material. It had problems and issues in terms of taking platelets and resuspending them and loss of platelets on transfusion. We have talked about -- Raleigh talked about the use of plasticizers that don't allow red cell storage for as long.

What are the other problems that you are not seeing that we have not seen with DEHP plasticized vinyls? If we do have a new plastic, it will have to be studied, I think, as extensively and be able to demonstrate the same level of safety as we have now with DEHP plasticized vinyls. And then finally, are the resources that we are spending on this to look for new materials -- are they actually not really spent elsewhere more effectively?

So, again, to be sort of provocative, do we

want to waste resources in looking for the idea? And once again, what is the ideal? And then who will pay for this and what is the real benefit? Once again, Joy indicated that Baxter has a plastic that has been approved and nobody really wants to buy it in the States. It costs more money and what is the real advantage. And then really is this really any longer a useful area of research and a useful area for new product development? I would kind of throw out to you, relative to the medical concerns that we are faced with, to spend a lot more money in this area to find the ideal material may not be cost effective. I throw that out and we can discuss it later. Thank you.

MR. HWANGBO: Thank you very much, Dr. Miripol. Now, speakers please come to the table for our questions.

DR. SNYDER: Yes. Ed Snyder from Yale. I would like to ask a question of the panel. Perhaps you can answer this question. In doing the research I did for the talk I gave, I came across what Dr. Ishikawa from the Japanese Red Cross talked about, this glow discharge treatment. And what it says here is that it is a radio frequency, 110 kilohertz, 800 watts, 9 second discharge under reduced pressure with carbon monoxide and argon, which apparently forms some kind of a cross link on the

DEHP surface which prevented leaching. If it turns out that DEHP and PVC are good, is there any point -- is this a proprietary manufacturing step? Is there some modification that might decrease the amount of plasticizer migration so we maybe can have our cake and eat it too?

DR. MIRIPOL: Well, that is a very good question, Ed. Yes, I think that there are some possible ways. Again, I mentioned briefly that we have reduced to some degree the amount of DEHP that extracts from our materials. But we still have to have DEHP there getting into the final blood component, at least the red cell. It is really not necessary for plasma and it is not necessary for platelets obviously. So certainly we are, from a manufacturing standpoint, looking at some of these methods and techniques. I can't tell you whether they are going to be cost effective or not. Because one of the issues, at least early on, is that some of this cross linking ends up changing some of the plastic properties, and that is not so good.

DR. CARMEN: I can add to that. The amount of DEHP needed to stabilize the red cell membrane is quite a bit lower than what is actually extracted. I am pretty sure there are films used in Australia that use a blend of plasticizers -- TOTM plus DEHP, for example. And

the amount of DEHP extracted is much lower. But I wouldn't fool around with this glow discharge stuff, I can tell you.

PARTICIPANT: Could the panel discuss this Excel or the laminated bag that is used in hospitals? Would that help?

DR. MIRIPOL: I think what you are talking about is a bag that is used for IV solutions.

PARTICIPANT: Correct.

DR. MIRIPOL: And it does not, I believe, collapse in the same way that vinyl blood bags collapse. I don't believe it has the same properties, but I am not really up on the specific chemical properties.

DR. CARMEN: Yes. We have had some experience with this, or at least one of the components. It is a polyester, but it is actually a co-polyester. It really does have some interesting properties. But we had to abandon it for several reasons, cost particularly.

PARTICIPANT: But many of the hospitals use that bag now, do they not? They have gotten away from the PVC and they all use the Excel or laminates.

DR. CARMEN: I think they use it, but it has got a laminate, and it really doesn't have the properties for a blood bag. It does for the IV solution. I am sure it is fine for that.

DR. MIRIPOL: I think one more comment on that. I don't believe it has that large of a market share either. I don't know if anybody in the audience -- I don't think it has -- you know, I think mainly because of cost I don't believe it has the market share of some of the other IV bags.

PARTICIPANT: I don't agree with that. I think people have moved all the way from PVC into the laminates or something that is other than PVC.

DR. SHEA: Hi, I am Katherine Shea. I am one of the afternoon panelists from North Carolina. I was just curious about your customers. As I was listening to you talk, I was just wondering if the customers that are not willing to pay the 10 to 15 percent extra -- this is for Joy -- are the hospital purchasing agents or if they are the physicians who are making the choice and then sort of directing the hospital purchasing agents?

DR. ANDERSON: Actually, our customers for blood packs are blood centers.

DR. SHEA: Okay.

DR. ANDERSON: Who are collecting blood and processing components. Those are the customers that I was referring to when I said that they perceived the products as basically being comparable.

DR. SHEA: And those are usually sort of adult blood banking professionals?

DR. ANDERSON: Yes.

DR. SHEA: Okay. So there isn't sort of a heavy pediatric representation in that customer pool? Just curious. I mean, so they are the people that run the blood blank?

DR. ANDERSON: They are the people that run the blood centers that collect and process blood into the components, and then they in turn supply it to physicians who are head of transfusion services at hospitals throughout the U.S.

DR. SHEA: But the decision is made before then? It is made at the collection centers?

DR. ANDERSON: Right.

DR. SHEA: Thanks.

PARTICIPANT: Stuart Zimmerman, FDA, cardio renal drug products. I was wondering to what extent there might be some interplay with the pharmaceutical companies in terms of possibly trying to find potential unwanted interactive effects. Because apparently there are changes in the body with these. Is there this interface or am I in the wrong forum here? Any comments?

DR. MIRIPOL: I am not exactly sure what you mean by changes in the body. If you would like to

elaborate.

PARTICIPANT: Well, in cells. You know, we have heard a lot about the potential effects of the oxidation process and proliferation effects of these cells. While we are getting into biochemistry, the drug companies are coming up with all kinds of new agents now. So one wonders if there is any potential synergistic effects between new drugs and their mechanisms and some of these other things. Like in the pamphlet here I read, the lungs is one concern and cardio muscle. Well, a lot of drugs are being delivered through the lungs now. They have all these new initiatives underway to deal with the lungs as a delivery system, for example.

DR. ANDERSON: I think one of the things that is important to remember -- because you elude to some of the early rodent studies and the toxicology studies -- is that those studies, as was addressed this morning, were oral feeding studies. And the metabolic properties in the peroxisome proliferation that is apparent in rodent models is not the same mechanism that is active in humans receiving IV blood products. So some of these animal models, as people eloquently discussed this morning, are really not directly applicable to the situation of patients receiving blood components.

DR. MIRIPOL: And also I would say that Dr.

Karle's talk I think spoke well to that. In other words, the actual studies in preemies undergoing ECMO. That is probably the most relevant human work that I know of.

PARTICIPANT: So there is no relevancy between drug interactive effects that you can envision?

DR. MIRIPOL: I don't know that that specific aspect has been studied at all.

PARTICIPANT: My name is Mark Mitchell, and I am with Mitchell Health Consultants in Connecticut. I missed part of the presentation, but I wasn't sure if you talked about -- you were talking a little bit about plasticizers as stabilizers or to be used as stabilizers for red blood cells. And I was wondering if you had talked about other types of alternatives to stabilize -- you know, other additives that may be used to stabilize instead of plasticizers.

DR. CARMEN: We did not, but there are such chemicals.

PARTICIPANT: Can you talk briefly?

DR. CARMEN: Well, I mean there are -- the literature is full of agents. I think Siegert did most of the work that will convey a protective effect on the red cell membrane.

PARTICIPANT: Okay. So there are other alternatives for stabilization?

DR. CARMEN: But that would --

PARTICIPANT: Okay. Thank you.

DR. CARMEN: -- lead to other problems.

PARTICIPANT: Hi. I am Kenneth Green. I am with the Reason Public Policy Institute. I was wondering if you know of any correlations between essentially the increasing cost factor of healthcare and the availability of healthcare. Because there is a break point at which the companies producing these products will have to pass the cost on to the consumer. And we know that inflation of healthcare costs is already a problem and is already keeping people out of the system. The question is where is the tradeoff? How many people are at risk or whatever or however small the risk is versus how many people would be put at risk by moving to a more expensive substitute? Do you all know of any information on that regard?

DR. MIRIPOL: Sorry.

DR. ANDERSON: No. I think I will make a comment, which is not exactly what you are asking but relevant, I believe. And that is that I think we should also be aware that some of this discussion and the implication that DEHP plasticized materials are somehow not safe has an implication for public health and does a disservice. For example, we are aware that some people who have been donors are now believing that they are at

risk from donating blood. If you ask, I think, anyone who is running a blood center in transfusion medicine, the prediction is over the next one to two years that there will be a shortage of red cells. And so to have donors believing that it is now unsafe to donate blood products is also a cost to the public health, I believe, that we really haven't considered.

PARTICIPANT: Thank you. I agree. The scare factor that would keep people away from donating is one. I did leukophoresis. I gave platelets when I was a poor graduate student -- actually, an undergraduate student. Therefore, I have received more than my share of phthalate exposure, I am sure. But still, I would do it again, since I chose eating over whatever risk there was.

DR. MIRIPOL: Let me also make one comment on that. I think the actual dose of phthalates that you may have received is probably far less than you probably expect that you received. I think as the recent literature would show, the process of donation -- on leukophoresis -- I think I had one slide on that and I want to comment on that -- is probably giving you a dose in the range of a microgram per ml to 5 micrograms per ml at the outside.

PARTICIPANT: I would have expected it to be more. Because they removed about three bags worth or

three units of blood and then put it back in you, all the time passing it through yards and yards of tubing.

DR. MIRIPOL: That whole process of DEHP extraction requires some time as well. It is not instantaneous.

PARTICIPANT: That is true.

PARTICIPANT: I might address the question of cost. Herb Cullis, American Fluoroseal. We did produce plasticizer-free bags made of teflon. They did cost three times the amount of the Baxter bags, and they were not saleable. So we could not sell them. And that maybe gives you an idea of the idea of the effect of cost. I think when you consider that there are about 12 million blood donations a year and perhaps three bags associated with each collection, cost is important.

DR. MIRIPOL: Thank you very much.

DR. CARMEN: Thank you.

MR. HWANGBO: I would like to thank you, our speakers. Thank you very much. And I would like to also thank our audience for the attention. It is 2:40. We are going to have a 15-minute break. So let's meet at 2:55, please.

(Whereupon, at 2:40 p.m. off the record until 3:02 p.m.)

CHAIRMAN VOSTAL: Maybe we can get started

and stay on track. Could you please take your seats? While you are taking your seats, I would like to take care of a couple of business items. One is that for the speakers and panel discussion members, if you have any reimbursements, there are forms available at the desk outside. So you can fill that out and get some reimbursements for cab fares and such.

The other point I would like to bring up is a mistake that occurred when we were advertising this workshop. There was a misprint that went out in the flyer, and it stated that the American Red Cross was actually sponsoring this workshop. That is not true. This is sponsored by the Center for Biologics and the Center for Devices, and the American Red Cross did not sponsor it or sanction the plasticizer workshop.

We are going to have a very interesting session coming up. As we have seen today, a great deal of studies have been presented. A number of them date back 20 or 30 years. So the problem is how can one take all these studies, maybe 500 to 1,000 studies, and boil them down into a message that could be easy to understand -- safe or not safe. The short answer for that is that it is extremely difficult. However, this year we are fortunate. There have been three independent agencies that have attempted to do this. They have looked at the

published literature and have done a risk assessment on whether DEHP and phthalate plasticizers pose risks to human health.

They are looking at the same amount of published literature. However, some of the outcomes were a little surprising that they came to different conclusions. So we were wondering how they managed to do that, and we asked them to come here and explain their risk assessment process.

So the first speaker that we are going to have today is Dr. Michael Shelby. He is the Chief of the Laboratory of Toxicology, Environmental Toxicology Program, NIEHS.

DR. SHELBY: Well, good afternoon. I do not yet have my head in the noose, and I will explain why in a few moments. First, I would like to introduce you to the NTP Center for the Evaluation of Risks to Human Reproduction. This Center was established in June of 1998. It is sponsored by the National Toxicology Program and the National Institute of Environmental Health Sciences. It is not a research organization. It is a data literature evaluation expert panel analysis organization that we put into place in the hopes that we could provide what would be considered scientifically rigorous, independent and timely evaluations of the

information that might be available on various chemicals to which people are exposed and might pose a hazard to reproduction or development.

To follow up on the Chairman's comments with regard to the amount of literature available. It is interesting. I think the field of toxicology, maybe like all other fields of science, appears to be rather inefficient. To have 500 or 600 publications available on DEHP and another 300 or 400 available on other phthalate esters and still have all the lingering questions seems to speak to the fact that somehow the right studies weren't being conducted through the years that those studies were carried out. But that is the way it is with benzene ionizing radiation and whatever you want to think of - dioxins. Somehow, after hundreds and hundreds of studies, we are still left with questions. So any conclusions that are reached, whether they be by the three groups that he mentioned earlier, that don't agree I think is generally a reflection of those gaps in our knowledge.

But on to the Center. The purpose for the Center is to, as I mentioned, to provide scientifically rigorous, unbiased timely assessments of the available information on reproductive risks, and reproductive including developmental risks for the Center. To present

these conclusions of expert panels to the scientific community, to government agencies if they are interested, and to the public in terms that are understandable to the public.

As I go through my talk, one of the slides will speak to the criteria that we use to select chemicals for evaluation, and one of those is public concern. It is an issue that may not be scientifically valid. It may or it may not, but it is certainly a valid social issue and one I think that government agencies such as ours have a responsibility to respond to.

And finally, to identify critical data gaps and specific research needs, which speaks to the topic I mentioned earlier that we don't always put our money where it is most needed. If we can clearly identify the types of research or testing that needs to be done, perhaps a limited amount of additional funding can lead to a great reduction in the uncertainties that are associated with the conclusions we reach about health risks.

The product that we anticipate for this Center is a report that provides the opinion of this expert panel on the scientific strength of the evidence that a particular exposure actually poses a hazard or rather a risk to human reproduction health and to the

health of our children. These monographs will be published in Environmental Health Perspectives, The Journal of NIEHS, and will be available on the Center's Web site. I have got a slide later on that will give you the address on the Web of the Center's Web page, and that these documents will be presented to the appropriate regulatory and health agencies within the state and local and federal government.

The structure of the Center is outlined here. The Center itself is made up of scientists from NIEHS as well as from a contractor who runs the central office for the Center. That is Sciences International in Alexandria, Virginia. John Moore and Toni Chalet are the principle investigators on the project. They are at the contractors. Their support staff, again both at NIEHS and at the contractor. We have got a core committee that oversees the daily or monthly operation of the Center, and this is made up of people from various government agencies that participate in the National Toxicology Program. There is an expert registry, which currently contains names, addresses and expertise of perhaps 250 scientists that represent a broad range of disciplines from toxicology to pediatrics to statistics -- a whole range of expertise that we anticipate will be useful in evaluating the literature on these evaluations that we

do. From that registry is drawn the expert panels, 12 to 15 individuals, who participate as independent scientists in these evaluations. As I showed you earlier, they produce the monographs which then go onto the Web site and into the journal, and those are distributed to the public, government agencies, and the scientific community.

The NTP Board of Scientific Counselors provides oversight for the Center with regard to its process and priorities. And finally, there is a chemical nomination process that is open to anyone -- anyone that feels that they have a candidate chemical or exposure or group of chemicals that are worthy of evaluation. That can be done through a telephone call, a letter, preferably over our Web site, where there is a page that permits you to put in some information that is useful to us. And then all nominations receive consideration. Again, this is just a list of things that are supposed to represent anyone and everyone from individuals to organizations and government agencies.

This is our Web site slide -- cerhr. I have been told I should have spent more time and applied my creativity to coming up with a name that yielded an acronym that would be more memorable than this, but that is all we've got -- cerhr. niehs.nih.gov. There is more

information on this Web site than simply specific items about the Center and its activities. One thing that we have really tried to do is provide links to other -- that will provide answers to other questions other than chemicals and the risks they may pose to human health. Any time you are dealing with reproduction and fertility and all those little key words, people are going to stumble into this site looking for other things -- assisted fertilization, socially or sexually transmitted diseases and that kind of thing. So we have got a bunch of launch pads for people that come in here. It is an interesting Web site. Take a look if you've got a chance.

It has got a description of the Center. It provides a mechanism for communication with the Center. The chemical nomination process is in there. The activities of the Center and full reports and summaries, which we are yet to have one, but we are working on one. Links to related sites, which I was just mentioning. And information on pregnancy issues of general concern. These are the kinds of things that we will probably not be evaluating, but things like cigarettes smoking, whether it is safe to have a glass of wine on Friday night if you are pregnant. What happens if you get measles. And there are a lot of issues that other expert

bodies have already addressed, and we have tried to provide you with information on those questions. And finally, in a closed section, we have got a communication mechanism for the Center, the core committees and the expert panels. That is not on the public side of the Web.

The chemical selection criteria, I just mentioned briefly earlier. These are four of the major issues that we consider. One is production volume. The second is human exposure as a chemical or a class of chemicals present in the environment or in products to which the product are exposed. Hard data in existence indicating that this chemical may have reproductive or developmental toxicity. And finally, as I mentioned, public concern about a chemical or a chemical mixture.

The first six chemicals that we considered are given on this slide. They include arsenic, inorganic arsenic, boric acid, diethylhexylphthalate and related phthalates, ethylene glycol, monomethyl ether, methanol, and nicotine or nicotinic acid. The core committee considered we compile dossiers on these and studied them and deliberated and in the end selected phthalates for our first expert panel because of widespread exposure, high production volume, consumer concern and recommended review of phthalates, especially in childcare products.

So it met admirably, I suppose, virtually all of the criteria that we use in selecting chemicals for evaluation.

So we ended up not with DEHP alone, but with some additional phthalates. Here are the seven that are currently under evaluation -- DEHP, butyl benzyl -- you have heard about some of these today. Bob Chapin talked about some of them -- dibutyl phthalate, dihexylphthalate, dioctal, di-isononyl, and the di-isodecyl phthalate.

This group of compounds to varying degrees have been studied for reproductive and developmental effects. Several of them have, at least in rodent studies, given evidence of reproductive toxicity or developmental toxicity. You have already heard a couple of speakers talk about those issues earlier today. And so these are the seven that are currently under consideration by our phthalates expert panel.

The panel met in August of this year for two-and-a-half days. These are the members of that panel, and they represent a wide range of affiliations and a wide range of scientific disciplines. It was a superb panel. I think I and most people that were in attendance at that meeting -- these are open public meetings -- was impressed with the intelligence, the

dedication, and the process by which these panel members went about their business of evaluating these seven phthalates esters.

It was probably a mistake on my part and that of the core committee to select so many chemicals for the first round. It is highly unlikely that the Center will ever again choose to try to evaluate seven compounds in one panel. The magnitude of the literature is excessive. I think there is right at 1,000 total references that we are dealing with and many uncertainties, as you have heard earlier today, with regard to the effects and the exposure levels and the exposure regimens at which those effects are observed.

But we met in August. We did not complete our deliberations and come up with our final product. Another meeting is scheduled for December 15 through 17 of this year. This first meeting, the August meeting, was held in Alexandria, Virginia. The December meeting will be held in Research Triangle Park. As I say, it is an open meeting. You are welcome to attend if you wish. At that point, we hope to complete this process.

The bulk of the scut work has been completed by the panel, and that is probably represented in the first bullet up here, to review the literature and areas of expertise. So each of the panel members was provided

to the literature that was relevant to their expertise and were asked to read it carefully and critically and to provide a summarization of that to the other panel members. The entire literature is available to all members of the panel on request. That was completed. They all provided their summaries. So the 15 members of that panel that you saw in the previous slide have got the complete summary of the data.

Then at the meetings, the panel writes an integrated evaluation document, which actually distills all of that information down into a five or six or eight-page summary and points out the strengths and weaknesses of the various studies that have been review. They point out the research and testing needs. That was completed for three of the chemicals by the time we completed the August meeting. The other four -- those are being revised and the other four are currently being written and will be completed before the December meeting.

At that point, the panel will discuss and reach a consensus on the integrated evaluations. That will be the first part of our December. The last thing that will be done will be to develop consensus summary statements -- and this is virtually the bottom line -- on the reproductive and developmental toxicity of these seven chemicals that we are evaluating. An evaluation of

the animal studies that have been done and an evaluation of what we know about human exposure to these various phthalates, and how the toxicity data and the exposure data in humans informs us about what hazard or what risk there might be to human reproduction and development.

I think the Chairman this morning -- Ron Brown, is that right -- was talking about some assessments that he had carried out, and these are very similar. These are not in the strict sense quantitative risk assessments. They are instead a thorough evaluation of the literature and basically a qualitative assessment of what risks might be associated with exposure to these chemicals.

It is our hope that these documents will gain the respectability and the acceptance of the scientific community in all corners to the point that these documents can be taken by chemical manufacturers and by regulatory agencies and by public health advocacy groups -- whoever wants to use them -- they can accept them as a scientifically rigorous and thorough document and use those to proceed with whatever assessment of risk that they are interested in conducting.

Finally, produce a monograph, as I said earlier. I mean, this entire package -- not the summary of the panel members, but the integrated evaluations and

the consensus statements will comprise the monographs that result from the panel's deliberations. That is it. I cut two-thirds of my slides out. I will be happy to answer any questions, I guess, after everyone else has talked. But please give us a visit on the Web site, and if you are interested, give me a call. I think my address and phone number is in the list of participants. Thank you for your attention.

CHAIRMAN VOSTAL: Thank you, Dr. Shelby. The next speaker will be Dr. Daland Juberg. He is a toxicologist with the International Center for Toxicology. He will speak about the risk assessment done by the American Council on Science and Health.

DR. JUBERG: Thank you, Jaro. I'd first like to thank Jaro Vostal, the CBER, and the FDA for giving the American Council on Science and Health the opportunity to speak here today and to present to you for your consideration their deliberations and their process that they went through in evaluating DEHP in medical devices.

I'll go a step beyond Dr. Shelby in saying that since the publication of our or this panel's report, I felt like my head has been in the noose and actually someone has been ready to kick the chair out from time to time. But that is okay. I am a believer that

disagreement will move us forward. Certainly there may be some disagreement on what risks, if any, DEHP poses, but I think collectively today this will give the FDA some future thought and consideration as to how they approach DEHP in medical devices.

For those of you -- I did present some of this material at the NTP CEHR meeting in August. So for those of you that are sitting through this again, bear with me. Some of it is the same and some of it is different.

Let me briefly start by telling you who is the American Council on Science and Health. They are essentially an independent national consumer education consortium founded in 1978. They are concerned not only with chemicals, but with public health issues related to food, chemicals, pharmaceuticals, lifestyle, the environment, and human health. The Council is served by more than 250 physicians, scientists and policy advisors who review each Council publication and participate in educational activities.

I think it is important to point out the mission of ACSH. This might help you get some understanding as to where they were coming from during their deliberation and review. Their top priority is to help Americans distinguish between real and hypothetical

health risks. The ACSH aims to separate the leading causes of disease and death from the leading causes of unnecessary anxiety and tries to ensure that both individual health decisions and public policies are based on sound scientific evidence.

Two examples that will serve to give you the extreme. The ACSH, for a long time, has been a vocal advocate on educating consumers about the dangers of cigarette smoking. Conversely -- and I think we would agree that that is a leading preventable cause of disease and death. Conversely, they were amongst the first to distinguish and really point out that alar, a growth regulator in apples, really doesn't pose a risk to human health. This was a fear that started about ten years ago, and in fact regulatory agencies and other organizations since then have agreed to this view, and so forth. So what they try to do is to distinguish those large risks from the rather small, negligible risks.

Just briefly, I would like to point out a little bit about some of the ACSH publications, their process and their funding. All publications are internally and externally peer reviewed. In other words, they have a regular publication process. And before anything is published, they send it out to some of their policy advisors, just to make sure they are not going out

on a limb with unscientifically supported statements.

The particular panel report on phthalates was peer reviewed by three external scientists as part of the publication process.

A little bit about where their funding comes from. The ACSH does receive support from more than 300 sources and maintains a no-strings-attached donor policy. It really is committed to publications and positions that reflect valid and current scientific evidence and information. They, in fact, have lost funding over the years from tobacco company food divisions that have dropped funding once the Council came out with certain position statements and advocacy positions on the dangers of smoking. Similarly, they lost funding from a metal pipe manufacturer when the Council reviewed and defended the safety of properly used plastic piping.

Finally, they do take a public health policy position. They have taken an aggressive role in educating the consumer about, again, the dangers of cigarette smoking. They have also alerted the public as to the risks associated with alcohol abuse and the neglect of preventive healthcare.

Let me now turn the attention toward the review of DEHP in medical devices. Why did they undertake this review and for what purpose? Essentially

because of the concern over charges that certain flexible devices, these being medical devices, posed serious risk to human health. It was a charge that was worth investigating. They saw this as a worthy topic of evaluation. And because of its mission to apply sound science to public health concerns, the Council convened a panel of relevant experts to evaluate and report on the scientific evidence related to concerns over DEHP.

ACSH is based in New York City, and they certainly did not have the staff or the expertise to evaluate this type of topic. So what they did was to convene a panel. Just as Dr. Shelby pointed out his panelists, I thought it was important to show you some of the panelists that the Council convened. It was a rather larger panel, 17 members chaired by Dr. Koop. It was an international panel. We had a Canadian representative, Dr. Ron Brecher. We also had a representative from the Netherlands, Dr. Hans Konemann. Most of these individuals are from academic settings. We did have three consultants on the panel, those being Dr. Brecher, Dr. Shayne Gad, representing the medical device industry -- not the industry, but he had experience in medical device registration, risks posed by medical devices and so forth. So he represented that particular sector. Finally, Jim Lamb, some of whose work was referred to

early this morning. We needed someone to evaluate the reproductive toxicity of DEHP, and so he agreed to serve on this panel as well. John Higginson, one of the founding directors of IARC served and gave us perspective on the carcinogenicity of DEHP.

Why such a diverse panel? Why did we convene? Why did the Council need 17 experts? I think essentially as Dr. Shelby eluded to, this is not simply a toxicology problem. This concern involves a number of different fields. And so it was very critical to have a diverse panel and a number of different medical and scientific fields represented. The issue demands multidisciplinary review. Certainly it involves exposure, effects, extrapolation of toxicological effects, species specificity, different animal models, metabolism and many others. So that is why we needed to convene and get a number of different experts to look at this.

Finally, I put inter-panel challenge and discussion. As you can imagine with 17 individuals, there is a good healthy discussion. There was much debate and discussion amongst this panel during the six-month review and deliberation. I think this was healthy to the process. We needed to understand the concerns of the panelists and address those and also deal with them and

answer them if we could. If we couldn't, we needed to find that out as well.

So from a public health perspective, the charge to the panel was as follows. This was in the letter sent out to prospective panelists. If the use of DEHP in medical devices poses a risk to human health, then scientists must defend that conclusion and recommend intervening measures to protect public health. Or if the weight of the scientific evidence shows no association between human exposure to DEHP and adverse effects, then that conclusion must be communicated as well.

What was this particular panel's process for the evaluation? It nominally involved primary peer review literature and reviews. Panelists were not limited in their evaluation. In other words, they were actually encouraged and charged with looking at all the literature from their particular perspective. they did this over the course of the six months. They were asked to focus on those studies that are relevant to the human scenario, particularly in the use of DEHP exposure from medical devices. So this would involve IV exposure primarily.

I will not discuss the toxicology today. Obviously that has been done quite extensively earlier. A criticism of this panel's report is that we did not

cite certain studies that served as some of the source material for the concerns. That is in fact true. We did not cite every study that was looked at. This panel looked at many, many studies. You have heard of the hundreds that were involved. We simply could not look at every study and discuss it in detail in the panel report. What the panel did decide to do was to focus on those relevant studies using a relevant route of exposure and using species that were perhaps more similar to humans than rats and mice.

Regarding the process, it was incumbent upon me as a facilitator and consultant to this group to make sure that all panelists' concerns were addressed. So at each meeting, I took a poll. I went around the room and polled every panelist to understand the concerns and to understand issues or areas that they needed more information. Following those meetings, I would then supply those pieces of information to the best of my ability. We often went beyond what was available in the published literature. We attempted to get the most current information. We tried to get the David, et al., cancer study, and we got that during its prepublication. We got a study -- an exhaustive review by John Dool looking at peroxisome proliferation that was in press at the time. I attempted to contact Dr. Karle, and we

bounced back and forth and never did reach each other, but the panel was very interested in whether there were additional data on infants. So we did not just rely on what was readily available. We often went beyond the call of duty to get information that was not readily available.

I think this panel -- and I was not a member of the panel, as I am not an employee of ACSH. As I mentioned, I served as a consultant to that group for this effort. But what this panel would tell you, I think, is that this review served as a snapshot in time. Really what they did was to take a look at the information, and they truly believed that the study of humans should be on humans. And if not on humans, then it should be on an animal model that is next closest to humans. So I think that is maybe a critical distinction in some of the differences that this panel came out with from those of others.

In any event, consensus development was important. Following the six-month process, if the panel could reach consensus, that was the goal. If it could not, that was an option as well. They did reach consensus ultimately, and that is in the published report.

Finally, what they wanted to do was to

prepare a public health statement for public consideration based on their review. You have heard from some of the researchers and other investigators this morning. There is much current ongoing research, and that is certainly a very encouraging sign. I think that is only going to add to what we know about DEHP and the risks or the lack of risks that may be associated with its use.

As I mentioned, a number of areas were evaluated during this process. The history and use of the DEHP, the human evidence for adverse effects, route of exposure was critical in terms of distinguishing and determining the relevance of toxicology studies for humans that are exposed during medical procedures. Exposure assessment is obviously very critical to this process -- metabolism, published risk assessment, some from regulators and some from other countries. This panel looked at a number of those. They took into consideration some of the risk/benefits associated with DEHP, and I will talk a little bit from the clinical perspective when I finish up about some of the benefits that some of our clinicians felt very strongly about. Finally, we had some discussion on alternative materials and whether there were readily available substitutes for DEHP if it were to be completely replaced tomorrow.

Certainly the toxicology of DEHP is very relevant and is a concern to this issue. In that respect, this group looked at carcinogenicity, genotoxicity, reproductive toxicity. They discussed some of the cardiopulmonary effects which have been reported. They also looked at liver and kidney toxicity and in addition looked at toxicological mechanism.

Before I move on and begin the latter half of the talk regarding the findings and some of the considerations, let me present to you, I think, what are some of the strengths of this panel review. In effect, it was an independent investigation. This was not done on behalf of the regulatory community. It was not done on behalf of industry. It was not done on behalf of trade associations or anybody else. The Council simply thought it was time to take a look at this issue, which obviously has been a concern for a number of years but which has heated up over the past two years. It truly involved unbiased, objective experts. In fact, in the letter that was mailed out to prospective panelists, it was a requirement that each panelist not have previously made any public statements or come out with any advocacy positions on DEHP. We did not want those kind of people on the panel that had already formed a preconceived idea as to the risk or lack of risk associated with DEHP.

As I mentioned, and I think goes without saying, it did involve a diversity of experts and disciplines. It was important that we not leave stones unturned. It did involve a weight of scientific evidence review. It didn't just look at toxicology data or exposure data or the lack of effects in some humans. It wasn't enough just to do that. We needed to look at all of these areas.

Finally, this report has been peer reviewed externally and it has been published.

Now, some of the considerations that this panel focused in on in forming some of its findings and conclusions. DEHP, in their evaluation of the literature, has been used safely in medical device applications for 40-plus years. We do have an extensive toxicological data base. This is not to say we have a perfect data base. In fact we don't. Nor do we, I would say, for any chemical. But we do have quite a bit of data. I am glad to hear that there is additional data forthcoming and that should help us out as well. But we do know something about the toxicology of DEHP. There are holes, but this panel felt that despite some of those holes, it did have enough information to reach a conclusion about DEHP. It did note that it was interested in the dose levels that are required for the

manifestation of certain effects. In other words, some of the toxicology literature will lead you to the conclusion that high doses are needed to reach certain effects. This is not true for all effects. But many of the studies, there is a critical dose level required. This is also to say that for some of those effects, we do see no observed effect levels or threshold levels below which toxicity is not manifest.

Route of exposure is very critical, and I think this is another important distinction. In medical applications, IV tends to be the predominant route of exposure. So from discussions you've heard earlier, there is substantial metabolic differences and metabolism differences, not only between species but in how people metabolize DEHP following oral versus IV exposures. This was critical to the panel's deliberation. Finally, I think interspecies differences do exist, and this is probably not a point of contention. There does seem to be some predominance towards a rodent sensitivity to certain DEHP effects. There tends to be some non-human primate insensitivity to certain effects. This is not to say that primates are resistant to all effects. It just means that animal model selection is very important in terms of extrapolating a certain effect to humans.

So what were the chief panel findings

following this qualitative risk assessment review. They found no human evidence of adverse health effects. They did note chronic toxicity is observed in some species following oral exposures that are above estimated human exposures. They noted in particular that IV administration in humans bypasses a critical enzymatic process, that is, hydrolysis in the gut, related to production of MEHP that is critical to certain toxicities. Human plasma DEHP levels during short-term medical procedures may approach some LOELS from long-term oral animal studies. However, human data have not reported effects following IV exposure. Obviously, we would like to have more human data, if possible. And if those are forthcoming, we would like to see those.

Finally, and I think this is a very important point, there are important species route and mechanism-specific factors that preclude direct extrapolation of animal toxicological data to humans. While the animal toxicology data is very important and while it can be useful to us in a risk assessment process, I think you cannot -- and this panel will support you cannot directly extrapolate rodent data to humans. The panel essentially felt there were more relevant studies which do not support a basis for concern over DEHP in medical devices.

So to conclude, some of the chief conclusions of this particular panel. There is no human evidence related to DEHP exposure after 40 years of use. Toxicological studies more relevant to humans do not support a basis for concern. We had certain clinicians on the panel that I think felt very strongly about some of the benefits from the use of flexible devices in the clinical setting. So this panel wanted to at least take a look at those. And for the benefit of the public health and for benefit of medical care, they wanted to mention and emphasize a few of these particular benefits that flexible devices afford medicine. This is not to say they have to be DEHP plasticized, but flexibility is a critical key here. We had clinicians that talked about long-term IV therapy as very dependent on flexible catheters. Needle therapy over the short term is okay, but needles cannot be used. They are injurious to the vessels and they cannot be maintained in place for long-term therapy. The procedures involving colonoscopy and esophagoscopy are very dependent on flexible devices, and the panel felt -- certain clinicians felt very strongly and had personal stories about the thousands of lives that have no-doubt been saved through the use of flexible devices such as these in terms of detecting previously undetectable tumors. Patient safety and comfort has been

increased obviously with these two procedures. If you had an inflexible device, it would be less comfortable. And finally another example is the treatment of hydrocephalus is very dependent upon the existence of a soft catheter.

So what did this group report based on their own considerations and deliberations and what they considered to be the essential and relevant data involving their scientific judgment as to the fundamentals of this overall issue. What they concluded was that DEHP, as used in medical devices, is not harmful to humans even under chronic or higher than average conditions of exposure. DEHP confers considerable benefits to certain medical devices and procedures and its elimination without a suitable substitute could pose a significant health risk to some individuals, these being ones that are very dependent in a clinical setting on the use of a flexible device.

The panel did conclude with a few recommendations, and I will leave you with those. Presently, DEHP-containing medical devices should not be removed from the market. Because of their critical importance to certain medical applications, DEHP-containing devices should remain available for patient use and to ensure patient safety. Finally, any

substitutes that may come along, and undoubtedly there are some, they should be evaluated in terms of their demonstrated function and reliability and also a risk assessment based on their toxicological profile and exposure data. As was pointed out this morning, are we moving from a "devil" to another devil, or from one potential risk to another potential risk. I think if we do move from DEHP, it is incumbent that there be an adequate toxicology data base equally as strong as DEHP, if not better, and there be demonstrated function.

So that is essentially where this panel concluded, and during the discussion, I will try to answer any questions I can on behalf of the panel. Thank you.

DR. NESS: Thank you, Dr. Juberg. The last speaker in this session would be Mr. Joel Tickner, who is a research associate at the Lowell Center for Sustainable Production at the University of Massachusetts. He will present the risk assessment done on behalf of Healthcare Without Harm.

MR. TICKNER: Good afternoon. Like Dr. Juberg, I would like to thank and commend FDA for holding this meeting this afternoon to have some open debate on the health risks posed by diethylhexylphthalate in PVC medical devices and the discussion on possible

alternatives.

Before I begin, just a little background. About a year ago -- actually about a year ago today, the Healthcare Without Harm campaign approached my institution, the Lowell Center for Sustainable Production, to look at and review the health risks posed by diethylhexylphthalate in PVC medical devices. The Healthcare Without Harm campaign had already been concerned about these medical devices in part because of the issue of dioxin creation and incineration, but also started looking at some of the literature about the possible health risks posed by diethylhexylphthalate leaching from these devices.

Just a little background on the centers. The Lowell Center for Sustainable Production is a research institute at the University of Massachusetts, Lowell. We are located in the Department of Work Environment. We are dedicated to developing, studying and promoting environmentally sound systems of production, healthy work environments and economically sound work organizations. We conduct research, training and outreach to government, industry and advocacy organizations. So we do work with all three.

The Healthcare without Harm coalition is a broad-based coalition of more than 200 organizations

dedicated to preventing pollution from healthcare settings. Some of the members of Healthcare Without Harm include the American Public Health Association, the American Nurses Association, the American Oncologic Nurses Association, and many environmental groups. This is just not a Greenpeace campaign. This is a broad-based coalition of healthcare professionals, advocacy groups and trade unions.

Let me start off with the key points from our review. First of all, and most important and not a trivial point, is lack of evidence is not evidence of lacking. This should not be confused with evidence of safety of DEHP. This is an important point and something we teach in basic epidemiology courses, that lack of evidence is not evidence of lacking. There is little or any human evidence to demonstrate the safety of PVC medical devices and DEHP over the past 40 years. This is an important point. As a result, we must rely on the toxicological evidence, because there is very limited human evidence. In fact, if we get that human evidence, that means we have failed as public health scientists. If we get human evidence, that means people have been harmed.

Second, DEHP is toxic to multiple organ systems, the critical effect being testicular toxicity in

the developing organism.

Third, DEHP exposure is highly variable, although some subgroups have significant exposures, and we will go through some of that in a minute. Hydrolysis to monoethylhexylphthalate, the putative toxicant, is qualitatively similar between species and route of administration.

And lastly, we will talk about the relevance of the mechanisms of toxicity in humans. There has been quite a bit of debate about the relevance of what is called peroxisome proliferation to humans, and that is relevant to carcinogenicity of DEHP, but much less relevant to the other toxicological endpoints.

To start off, our methods. We undertook a literature review of both in vitro and in vivo DEHP exposure, metabolism and toxicity data for the period 1945 to 1999. Our goal was to include references that represented the full spectrum of data. We didn't feel like we had to have the full data base, just like the other committees and other researchers, but we felt that we should be comprehensive.

The next step after doing an initial literature review was to undertake bibliographic searches based on those references and interviews with primary DEHP researchers to better understand the uncertainties

and debates within the science. Our criteria for inclusion in the study was, one, addressing an important aspect of toxicity, exposure and metabolism of DEHP, addressing species differences in metabolism and toxicity, addressing age-related toxicity, addressing toxicity in multiple organ systems, and examination of key uncertainties.

Our next step was to do market research on alternatives, including technical literature, Worldwide Web searchers and interviews with manufacturers. Like the other committees, we weren't trying to calculate an exact risk number, but to understand the risk to human health.

Within this, I am not going to talk about individual studies, but just to note that the vast majority of the literature on DEHP is rodent literature. There is just a handful of primate studies on DEHP toxicity.

Evidence in humans. As I said, there is limited evidence of human effects from case reports, and there is limited evidence of no effects from case reports. But it is quite limited. As I said, there is no population-based epidemiologic studies, despite these recommendations in an NIH-sponsored report in 1975. This report on diethylhexylphthalate from PVC medical devices

recommended an epidemiologic study of dialysis patients to understand whether DEHP posed a risk, and that study was never undertaken.

Even if such studies were undertaken from an epidemiologic standpoint, they would be quite difficult to conduct for many reasons. First of all, the long-term follow-up of high risk groups is quite difficult. These are ill people to begin with. And following up over the long-term is quite a difficult task in epidemiology. We have long latent periods between exposure and effect for some of the toxicity endpoints, subtle effects for some of them, quite a big variability in both exposure and susceptibility within the human population, and a ubiquiative exposure. Diethylhexylphthalate and the phthalates in general are among the most ubiquitous contaminants in the environment.

So Dr. John Karns, the eminent ecologist, in Environmental Health Perspectives a couple of months ago wrote in an editorial, "While high uncertainty may obscure both the probability of a risk and the magnitude of harm, uncertainty does not eliminate risk."

In addressing DEHP disposition in metabolism, first of all DEHP is widely distributed to multiple tissues and crosses the placental barrier. I think you have heard this all day. DEHP is distributed

to the lungs, the kidney, the heart, the liver, and several other organs including the fat. Primary metabolism to MEHP, which is the toxic metabolite, appears to be qualitatively similar between species. In other words, different species metabolize -- all of the species identified metabolize DEHP to MEHP, though there are differences. And you could say there is probably a difference in risk between a rodent and a human. However, there is little known about the secondary metabolites -- very little in the literature about the secondary metabolites of DEHP. And this is where there is really differences between rodents and primates and humans is in secondary metabolites and metabolysis.

Second, ingestion appears to result in greater formation of MEHP, and this is an important but -- MEHP has been measured in stored serum. The lipases in stored serum convert DEHP to MEHP, and in neonates and adults. Several studies have shown that. Pollock, et al., showed that equal concentration circulating of MEHP and DEHP in dialysis patients after IV DEHP exposure, and Albru, et al., measured MEHP as 18 percent of total metabolites in urine after transfusion. So in humans, DEHP is being converted to MEHP after intravenous administration.

There are also age-related differences in

metabolism. For example, neonates have a much lower ability to glucuronidate MEHP into its secondary metabolites, meaning that there is a possibility that they have a longer retention of MEHP.

Finally, and most important about disposition in metabolism is that animal studies using the oral route will provide important insights into potential IV toxicity to humans, because we find in intravenous administration that conversion from DEHP to MEHP.

DEHP toxicity. Our review found that DEHP is toxic or toxicity has been observed in the liver, the reproductive tract -- the testes, ovaries and secondary sex organs -- the kidneys, the lungs, the heart and the fetus. Again, testicular toxicity appears to be the critical effect. It happens at a much lower dose level than most of the other effects. However, we found in the literature that effects to the heart, lungs, liver, testes and kidney have been observed in laboratory studies or in limited human case reports at levels of exposure that may be experienced in certain clinical settings. As I said, for some adverse effects, such as testicular toxicity, the developing organism, fetus and neonate, is much more sensitive than the adult. And this is a critical point because most of the toxicological

literature is based on adult animals. So we have a very limited knowledge of what might happen in the developing organism.

This table, which should be in your handouts and you probably can't see very well, is just a listing of the different toxicological endpoints from DEHP. I am not going to go through them just because of the limited time, but that should be in your handouts. It is the literature that we have looked at, part of it. But what it does show is that some of these effects have been shown at levels that might be found in clinical setting.

Mechanism of effects. This has been really the area of contention, both that and the metabolism. And the mechanisms of effects is important because DEHP is what is called a peroxisome proliferator. Peroxisome proliferation appears to be necessary for the hepatic carcinogenic response in rodents, though the exact mechanism is still under debate. There is a literature examining exactly how peroxisome proliferation leads to the carcinogenic or hepatocarcinogenic response. But this is just really about hepatocarcinogenesis. Rodents have a much higher expression of PPAR alpha, which is the nuclear receptor necessary for peroxisome proliferation, than humans. Though peroxisome proliferation in the literature appears to be a dose and not species-dependent

phenomenon. In other words, there is literature that demonstrates that humans do undergo peroxisome proliferation. So it is more a dose rather than a species-dependent phenomenon. And the variability in the human population is fundamentally unknown. There is some literature to demonstrate that older animals undergo peroxisome proliferation to a much greater degree than younger ones.

However, this same research on what are called knock-out mice have demonstrated that non-hepatic carcinogenic effects occur at least partly independent of peroxisome proliferation. That means that these animals, which were bred without the nuclear receptor PPAR alpha, do exhibit effects in the kidneys, the testes and to the fetus, and these effects are at least partly independent of peroxisome proliferation. Thus, we concluded that the mechanisms of these effects are multiple and likely to be relevant to humans.

With regards to the carcinogenic response, the hepatocarcinogenic response, we found that there is still quite a bit of debate about the exact mechanism. It is not as conclusive as some articles seem to indicate. And as such, concurring with our results, the California Office of Health Hazard Assessment in early March issued a statement reviewing DEHP

hepatocarcinogenicity saying that we have reviewed this literature on peroxisome proliferation and we still cannot say that exposure is without a hepatocarcinogenic risk.

Exposure to DEHP. In the literature, leaching has been identified from IV bags and blood bags, tubing, dialysis membranes, and catheters during different procedures. The literature demonstrates that dialysis patients and high risk infants receive significant exposures for long periods or at critical junctures in development -- again, the issue here is critical junctures in development, the developing fetus or the developing neonate. What we did find in the literature is highly variable exposure. Some rationale for that highly variable exposure may be the device, the stored media, the storage time, the temperature and humidity under which it is being used, pressure and agitation, DEHP content, and study design itself among other factors. So there is this wide range of -- and surface area, I should say. There is this wide range of variable exposure that is hard to explain in the literature, but it is variable and it depends on many different factors.

As such, we concluded that there is really a difficulty in controlling leaching exposure amounts

without physical modification of the device. Exposure will be highly variable depending on many factors, and without physically modifying the device, for example putting in a secondary plasticizer or some kind of coating, you are going to have a really hard time controlling specific leaching and exposure.

Just to give you an idea of the ranges of exposure depending on the procedure. Hemodialysis, .01 to 7.2 mg per kg body weight. The real critical one is exchange transfusion and ECMO, which Schneider found 42 to 140. I know Karle found quite a bit lower. So there is this wide variability, but the high impact or the high risk populations would seem to be those hemodialysis patients and ECMO and exchange transfusion patients.

We also, in addition to looking at DEHP toxicity, started to look at alternatives to DEHP and PVC. I think the first point which is important, which I have heard brought up -- I came in late, but I heard it brought up several times -- is the positive aspects of DEHP in terms of leaching. That it does have a positive effect in stabilizing red blood cells. However, what we found was this couldn't be exactly controlled. So it is fairly uncontrolled leaching and variable and not regulated. Any other blood additive would be regulated as a blood additive. And we felt that in looking at this

that if DEHP is going to be a positive additive to the blood supply, then it probably should be regulated as an additive to the blood supply.

We looked at some of the polyolefin bags available for platelet-rich plasma, platelets, and fresh frozen plasma. Some of the polymers -- some of the others, silicone, EVA, and polyolefins and polyurethene for tubing. Polyolefin laminate IV bags are readily available and have up to a 20 percent of the market share. And there is this whole development in polymer innovation among the metallocene polyolefins which have distinct properties. We did make clear, though, that there is really a need for innovation in red cell storage and tubing. Red cell storage, as you all know, has no alternative to PVC other than using citrates as a replacement for DEHP. So there is that need to do innovation. What I found -- and we also at University of Massachusetts at Lowell as a leading polymer engineering school, and I found that you give a polymer engineer a task to do, and they will come up with a solution. So I think a lot of this is pushing innovation. There is a need to identify materials that meet existing performance requirements at reasonable cost, pollute less through their life cycles, and do not expose patients to potentially toxic substances.

In conclusion, our review found variable patient exposure to DEHP with significantly exposed subpopulations -- again, high risk neonates and chronically exposed dialysis patients and others. There is a lack of epidemiological studies and evidence as to the safety of DEHP or its hazards. However, there is a sufficient toxicological data base readily available to cause concern about the advisability of exposing the fetus, neonate, infant, child or chronically ill adult to DEHP. And also the current availability of some alternatives presents a compelling argument for moving assertively but carefully to the substitution of other materials. For applications where no alternative currently exists, we would recommend a dedicated research and development program encouraged and supported by FDA to help identify safer substitutes.

In closing, I will just say that our report has now been submitted for publication in several peer review journals, so it should be out in the scientific press relatively soon. Thank you very much.

CHAIRMAN VOSTAL: Thank you, Mr. Tickner. I would like to invite the speakers at this session to come up to the front for a question session. It turns out Dr. Shelby had to catch a plane and Dr. Bucher will step in for him.

DR. BUCHER: I will deflect any questions that Mike would have deflected.

CHAIRMAN VOSTAL: Actually, I was going to ask Dr. Shelby a question. I was wondering whether -- we heard about some studies there in the works right now, the multigenerational toxicity studies. Will those be considered in the assessment of the Center?

DR. BUCHER: No. The only data that will be considered are those peer review publications that will be finished or that will be in the literature prior to the December date of the meeting.

DR. CHAPIN: Jaro, can I just make a comment about that?

CHAIRMAN VOSTAL: Sure.

DR. BUCHER: I didn't deflect it properly, is that right?

DR. CHAPIN: No. You did a fine job, Bob. Bob Chapin, NIHS. One of the points that Mike made about his Center's process is that it is an iterative process, and as new data are developed, the Center and its machinations are designed explicitly to be able to take advantage of new data. And while in part we sort of regret the way the timing on this has worked out, on the other hand it is a great opportunity for the Center to be able to use the iterative ideas there and kind of work

that into the process the first time through. So as the new data come out, I am sure they will be incorporated into subsequent revisions. As those data are available, they ought to be incorporated. Is that right, John?

DR. BUCHER: Yes, I will add to that. One of the purposes of the Reproductive Toxicology Center was to generate research needs. Hopefully, the National Toxicology Program would then step in and fill some of those research gaps, so that we could then answer the questions that were raised. But the December peer review will not actually have any of the data that were referred to in the two studies.

PARTICIPANT: Good afternoon. Kenneth Green with Reason Public Policy Institute. For the gentleman from Healthcare Without Harm. It is true that a tenant of epidemiology is an absence of evidence is not evidence of absence. But a fundamental tenant of all science is that you can't prove a negative. And what you are suggesting is that something must be proven safe, not that harm must be demonstrated. So I guess I would ask you, what exactly is the battery of tests that you think would lead to an outcome of proven safety? And can you name any single man-made chemical or substance that can pass those tests?

MR. TICKNER: Good question. Actually, I am

not from Healthcare Without Harm. I am from the University of Massachusetts at Lowell, but representing the report we did. I don't know whether that is an appropriate question for this discussion here about any chemical and its safety. Generally what we do is to determine in risk assessment whether a chemical might pose a health effect is to look at toxicological data because human evidence is rarely available. Of course, as you are saying, you can't prove anything safe. But you can't say because there is no evidence that the evidence is lacking -- that there is no harm. For example, there have been no epidemiologic studies in the literature looking at DEHP health risks in medical devices. Now that -- if there were negative studies, that would be a different thing. But there are no studies. And that is what was found at the NIHS panel as well.

PARTICIPANT: Okay. One follow-on then. Can you comment on the fact that we have a 30-year natural experiment in which despite increasing numbers of people exposed to phthalates, including in the most vulnerable groups you've talked about, you have an inverse correlation in terms of the rates of cancer and no trend in terms of testicular degeneration, infertility growth, sperm deformities -- nothing that has been substantiated. How do you respond to the natural

experiment indicating that it doesn't exist.

MR. TICKNER: Well, you wouldn't be able to tell from a global population data of sperm counts and cancer rates whether DEHP exposure from medical devices actually posed or caused a risk to humans. Just science can't do that. You can't take out that little piece from that big puzzle.

DR. CHAPIN: But in point of fact, there is an increased rate of hypospadias and testicular cancers that have been happening that have been reasonably well documented and I think are reasonably convincing. Now what those are due to, nobody has a clue.

PARTICIPANT: And has it been separated from detection and early detection?

DR. CHAPIN: Yes.

MR. TICKNER: And I should say, too, that that -- the hypospadias issue is one of the reasons that the National Centers for Environmental Health at CDC is looking very closely at DEHP exposure in the human population through urine, and they have undertaken a multi-million dollar, multi-year exposure assessment of the human population for phthalate mono-esters.

PARTICIPANT: Bob Rubin, Johns Hopkins University. A question for Mr. Tickner. Did I hear you state the peroxisomal proliferation has been demonstrated

in humans? And if so, which experiments?

MR. TICKNER: It has been shown, I believe, in some of the experiments looking at hypolipidemic drugs. There has been some evidence of peroxisome proliferation. There is a study by Ganning, et al., that they found peroxisome proliferation in liver biopsies of patients undergoing dialysis.

PARTICIPANT: With the fibrates, not with DEHP.

MR. TICKNER: With which?

PARTICIPANT: With the clofibrates.

MR. TICKNER: No, that -- yes, exactly. But peroxisome proliferation does occur in the human population is what that shows you. It shows you that humans are not naturally completely resistant to peroxisome proliferation.

PARTICIPANT: But I am not aware of any evidence that shows that DEHP causes proliferation in humans.

MR. TICKNER: Again, Ganning, et al., examined liver biopsies of patients undergoing dialysis and found that after six months, there wasn't peroxisome proliferation, but after one year they observed peroxisome proliferation in liver biopsies of patients undergoing dialysis using DEHP plasticide devices.

PARTICIPANT: But that is not evidence that DEHP did it.

MR. TICKNER: Well, that was their conclusion. And I must add, though, despite that, even if peroxisome proliferation is not relevant to humans, we know that these other effects occur at least partly independent of peroxisome proliferation.

CHAIRMAN VOSTAL: Joel, I have a question for you. In your center, is that -- the risk assessment was done by an expert panel or how was that conducted?

MR. TICKNER: It was done by myself with several other researchers where we worked with other researchers. But the main writing was done by myself and about four others. Then the report was submitted for both internal and external review. And any of you from academia can support me in saying that your colleagues are the toughest peer reviewers you can have. And I can tell you that it was as rigorous a peer review as any document I have done.

CHAIRMAN VOSTAL: Maybe a question for Dr. Juberg. Do you think there are any more studies that should be done, or do you think that we have actually answered all the questions in terms of DEHP toxicity?

DR. JUBERG: I guess -- as I said during my talk, I think the current ongoing research is very

interesting. It will be thought-provoking. It will particularly be interesting to see how regulators in the risk assessment community apply those studies to the current risk assessment of DEHP in medical devices. In terms of other ongoing research, I think our panel felt very comfortable with the information it had in terms of reaching the conclusion it had. So right now, I would say the answer would be no. Certainly if there were other human data that come to light, they would be very interested in that. But in terms of providing or proposing other additional toxicological studies, the panel didn't have any particular thoughts there.

CHAIRMAN VOSTAL: If there are no other questions, thank you very much. We will just move on to the next session, which is going to be the panel discussion. The panel discussion will be moderated by Dr. Mel Stratmeyer from the Center for Devices.

DR. STRATMEYER: If you think it is bad having to talk just before lunch, try talking about 11 hours into a meeting. First of all, up to this point I think everybody realizes that this workshop has dealt with scientific issues regarding the toxicology and efficacy of plasticizers in blood bags. You've also heard the perspective and the activities of various groups on the use of plasticizers in this context. This

particular panel, however, is represented by a wide variety of clinical disciplines, including transfusion medicine, intensive care and clinical toxicology.

The purpose of this panel discussion is to integrate the information discussed earlier today, and the panelists' general knowledge of DEHP and other plasticizers and to integrate this with the clinical experience to address the question of human risk from plasticizers in blood bags. Questions have been presented to the panelists to facilitate discussion. However, discussion pertinent to the issue need not be restricted to these questions, and although it is not listed in the agenda, I intend to open up the session to questions from the audience at the end. So don't go away, you will get the chance to ask the panelists some questions.

Now let me introduce the panel members. Please raise your hands, because I can't see all of the signs there. First of all, you have met some of them already. James AuBuchon from Dartmouth Hitchcock Medical Center. He is the Director of Blood Bank and Transfusion Service. Ed Snyder -- Dr. Ed Snyder. He is the Director of the Blood Bank and Aphoresis Service at Yale New Haven Hospital. And Dr. Naomi Luban, who is at Children's Hospital, Washington, D.C. She is the Director of

Transfusion Medicine there. Dr. Katherine Shea, a pediatrician associated with McMillan and Moss Research Incorporated. She is also a member of the Phthalates Expert Panel for the Center for the Evaluation of Risk to Human Reproduction. Dr. Scott Phillips, Health Sciences Center, University of Colorado at Denver. He is into clinical toxicology. And Dr. Peter Orris, with the Department of Occupational Medicine at Cook County Hospital. He is also a professor of preventive and internal medicine at Rush Medical College. And then Dr. May Jacobson, who is with Children's Hospital at Boston. Dr. Jacobson is a co-investigator of the study regarding the effect of DEHP on hepatic function in histology in monkeys.

So with that, I would like to go on to the first question, please. Our answers will be more interesting than the questions. If DEHP was removed from use in blood storage bags, what impact would that have on the availability of red cells for transfusion. And if DEHP was removed from use in blood collection sets including tubing, what impact would that have on transfusion practices. Again, I hope that with their clinical background, we will get a little bit of an idea of exactly what is going on with exposure to DEHP today.

DR. SNYDER: Well, I think the first thing

that -- the first part of this question that you have to answer is how quickly is this going to happen? If it happened tomorrow, it would destroy transfusion practice because we don't have anything to replace it. If there is a replacement that is available and the manufacturers can provide it, life would go on. So it is not so much a problem of loss of DEHP would be an adverse effect. It would be loss of the ability to provide transfusion period is the problem.

DR. STRATMEYER: Okay.

DR. AUBUCHON: If I could take a slightly different approach to answering that question, although I agree with everything that Ed has just said, if we were to attempt to replace DEHP with a non-leachable plasticizer -- TOTM, we have seen the data earlier today -- we would immediately see two changes in the storage of red cells that would have an impact on patients. The first, as I showed the data earlier on this morning, there would be about a 17 percent reduction in the number of available red cells in a unit that had been stored for its full storage period, 35 or presumably 42 days. And even in shorter storage periods, I would expect there to be a similar decline in availability, although somewhat less at shorter storage periods. That would have an impact primarily on chronically transfused patients, in

that they would need more frequent transfusion. For the occasionally transfused patient, for example a surgical patient, that may not make a huge difference, although in multiple transfusion situations a patient undergoing complex surgery getting six, eight or ten units, that may require them to need another unit. That would place an additional strain on the blood supply system at a time when we are very concerned that in the next year, the line of availability, which is decreasing, and the line of utilization, which is increasing, will cross. So, therefore, there could be shortage on that basis.

A similar outcome would be shortage due to decreased outdate. That is, I am sure the FDA would still require the same efficacy standard, that is 75 percent recovery at 24 hours after transfusion for any licensed blood collection and storage system. That would then necessitate, if we were to remove DEHP and replace it with something like DEHTM, a shortening of the storage time from 42 days to 35 or possibly even a shorter time period. That would inevitably lead to greater outdating. We certainly have seen that in the past that when we have lengthened the storage period for red cells, we were able to decrease outdating and we would probably see the opposite happen if we shortened the dating period. And that would also have an adverse effect on the blood

supply.

DR. LUBAN: From the perspective of infants, one of the recent and probably most current practices of neonatologists is to attempt to keep an individual baby on a single unit of blood until the outdate of that blood, the effort being to make donor exposure as minimal as possible and hopefully, at least for red cells, down to one donor exposure for the period of time that the baby is being supported through iatrogenic blood loss, which is in fact the major cause for most infants needing to be transfused. So clearly any replacement product would have to be able to provide us with something similar to what we have now moved to, and in addition to that, we would likely have to be repeating many of the studies which have just been published on the safety and efficacy of that practice.

The other point I would like to make is that you, in point A, discuss blood collection kits including tubing. And particularly for babies, the way the transfusion is administered at the end is very critical and oftentimes we are drawing blood up in syringes and infusing them through different kinds of specific infusion devices, which have yet another whole issue attached to them as far as leachability of plastics.

DR. STRATMEYER: Anybody else like to --

DR. ORRIS: Speaking as a generalist here, I was interested in the consensus from the morning's discussions concerning the fact that while the DEHP provides some interesting stability and use in the blood bags and to the red cells, it clearly leaches from the products, there seemed to be general agreement about that, and leaches in levels that are in the ballpark for what some of the studies have documented to be causing problems in laboratory animals. Based on that, I thought the interesting discussion was actually how far along the alternatives have come. That is what I was surprised about. I was surprised about the Baxter presentation and some others about how far along they were in developing of alternatives. Fortunately, this question is asked in a rather absolute and time current way, but the process is not such. So at least for myself, I am very stimulated by the fact that there is this exploration of alternatives and think that that ought to proceed, so that maybe we get out of the conundrum that are currently being presented. I am sorry, Peter Orris.

DR. JACOBSON: I would go along with what people were saying that you can't immediately change. I mean, the phthalates have a high degree of reliability. But I do think that there are alternatives that should be looked at. The Baxter people spoke about the citrate

plasticized bags, which could give you a 42-day storage in red cells, and I think this is where we should be going.

DR. SNYDER: Well, one of the concerns is that you may be able to change the bag, but I just made a little jotting here of the other parts of the transfusion community that probably use plastics that contain DEHP. In addition to the blood bags, the pooling bags, the transfer tubing, administration sets, the filters, all phoresis collection equipment including photophoresis, plasma phoresis, platelet phoresis, leukocyte collections, all stem cells and every bone marrow transplant that is done uses equipment that has plasticizers in it. And I would think one would need to evaluate the suitability of storage of a variety of things in these new plastics which may never have been looked at in the oncology setting as well. So it is something that could take years and years of evaluation. Admittedly, if you are doing a stem cell collection, it is not stored for 42 days because it is usually frozen. But again, the freezing process may require that DEHP plasticized products are part of the fluid pathway and what effect does that have on long-term storage and short term and so forth. So it is a very slippery slope as far as you can make one change, but it is like washing your

socks but nothing else and you are still not clean unless everything is clean. That was a very bad analogy, but it is what came to mind at the present time. Please strike that from the record. A partial cure is not what I am hearing everybody wants. People are trying to get the complete overhaul of the system, and I don't know if that would occur in the foreseeable future.

DR. PHILLIPS: Just a couple of comments. I think I would defer issues of transfusion practices, obviously, to the transfusionists on the panel here. However, I think as a medical toxicologist, I think that before we proceed to alternatives and sort of full-scale production use, we obviously need to have a significant amount more data available, particularly toxicologic data on people when we have a product that hasn't shown adverse effects in people. So I think the replacement products have to have a pretty darn good track record before we move in that direction.

DR. SHEA: I agree with that, and I would just add in response to you, Dr. Snyder, that one thing to think about is the vulnerable population. And if we do believe that the pediatric and neonatal population represents a potentially vulnerable population, which the tox studies certainly support in terms of reproductive toxicity, then you can significantly decrease exposure by

storing the blood for one month or for five weeks in a non-leaching container, even if you collect it in the DEHP. And then you might get the best of all possible worlds. You might get the red cell benefits and not the excess dose.

DR. LUBAN: I'm unaware, and please fill me in if I have missed it, any data that has looked at simple transfusion and toxicity from simple transfusion, meaning topper transfusions of 10 to 20 ml per kg over a prolonged period of time in babies. The data to date that perhaps gives some significance is on exchange transfusion, and I might add that exchange transfusions are usually done with the freshest of blood that has been stored for the shortest period of time, perhaps correctly or incorrectly, but with the theory that one gets a longer in vivo survival of that blood in the infant after the exchange transfusion. I also jotted down a few of the other things that an exchanged transfused infant is likely to have ongoing at the same time that might have leachability, which includes a pick line, an umbilical artery or a venous catheter, a feeding tube and potentially a CNS shunt catheter, and that infant might well also be exposed to some sort of a flexible scoping if the baby develops abdominal distention and necrotizing enterocolitis is considered. So I think we have to be

very, very careful when we deal with the removal of one element of the system to consider all of the other elements of the system as well.

DR. AUBUCHON: If I could add a practical viewpoint from really a non-medical issue, and that is cost. Clearly Baxter has had the unfortunate experience of putting a lot of effort into developing an alternative plasticizer and found it going nowhere in the marketplace. And that was at a time in the early 1990's, when although hospitals felt put upon and blood centers felt put upon in terms of the financial constraints, looking back those were the good old days. For a hospital transfusion service now to be faced with a new blood bag opportunity and a 10, 15, 20 or 30 percent increase in cost at the same time that it was being faced with similar increases in cost due to federally mandated changes in the blood system, whether that relates to geographic deferrals for CJD or new forms of testing to reduce viral transmission risks, it is all increased cost to the hospital and no increased reimbursement coming through the other end. As long as the phthalate plasticizers are not perceived as a real health risk and a replacement for DEHP is seen as gilding the lily, hospitals and blood centers will be entirely unwilling to spend any additional money on an alternative.

DR. ORRIS: I am really a little confused as to some of the reasoning of my colleagues who are involved in the very careful therapy for these very small children. You place a very high premium on the accurate evaluation of the drugs that you are putting into those children. You place a great reliance on the FDA to insist upon a high degree of security as to the effects of those drugs and the safety of those drugs and a variety of other aspects of those medications. We are now learning that we have a compound here that is being added to those drugs in significant quantities, so we are learning, and it is being added in variable quantities to those drugs, yet there seems to be a hesitation of placing the same criteria on that additive drug, if you will, or that additive chemical as you are placing on the chemicals in which it is being mixed. I am wondering why that is and what is the difference in this equation here, other than the economic, which is obvious I would say.

DR. STRATMEYER: Well, I think really we are starting to move a little bit into question 2 at this point in time. So as you get ready to answer it, Dr. Snyder, let me first put up question 2. Because I think you are going to find that we are starting to move in that direction.

As it has been said many times today, blood

containing DEHP and MEHP as a result of leaching from PVC materials and enzymatic conversion has been transfused for over 30 years with no documented adverse effects in humans. So the questions that this poses are how can the results of animal studies be reconciled with the lack of documented human effects, and are there subpopulations that may be at greater risks from exposure to DEHP in blood. Again, the question that Dr. Orris has raised sort of gets into that whole issue of your accepting the idea basically that there are no documented human effects, so therefore let's keep what we have got. He is I believe asking whether or not you have used the same criteria for DEHP as you would use for any substitute that you might use. Is that --

DR. SNYDER: Yes. I mean I think the concern is that for over 30 years, there hasn't been documented adverse effects -- substantive documented adverse effects. Dr. Luban, I am sure, somewhere in her institution there is a wall littered with children that have been successfully treated by the Children's Medical Center, all of whom have been exposed to these plasticizers and who have gone on to have probably children of their own and so forth. It is the weight of that evidence that makes us -- forgive me if I am speaking for you, Naomi, incorrectly -- that makes us

reluctant to change too quickly because there may be adverse effects from the other plasticizers and other products that we are not aware of yet. I think Dr. Anderson had a slide which tremendously impressed me and which I don't remember well enough to quote it, but it was something like a billion year history of exposure to plasticizers -- was I close -- without harm. Which I thought, based on the number of transfusions given since the beginning of time and the number of people and so forth, that is a very substantial record of lack of harm for such a large number. I think we would be derelict in our duty if we said, yes, there is a concern about this and let's consider changing. One thing I am getting very uncomfortable with is that lines are being drawn and it looks like the industry, and transfusion medicine specialists have been considered by the FDA at times to be part of the industry although we consider ourselves more academic -- that it looks like the industry and the blood bankers are stonewalling and saying it is too much trouble and it is going to cost too much and look at all the -- it is impossible of you to try to change this. I don't think we mean that at all. But I think the reality of the situation is that there are cost issues, there is public health issues, and this should be the beginning of a very long, prolonged debate and not something which we

should switch to, nor is it being suggested that we switch to it immediately. These are some very serious issues which perhaps this meeting would be the first clarion call that we need to reevaluate this again. I didn't see many transfusion medicine specialists in all the public advocacy groups that were listed, very scholarly individuals. But I think the medical community needs to play a role as well in this, and I am sure it probably will in the future.

DR. SHEA: I'd just like to say that we do have 30 years of experience, but we haven't been looking at some of the endpoints that we may be most interested in looking at. A lot of the early work was really concerned with the carcinogenic properties of DEHP, and I think the science of the reproductive toxicity is developing and changing rapidly. I think that this is sort of a misleading statement. So what I see is an opportunity that we shouldn't miss, which is that we are very likely going to continue to expose very sick infants or maybe not so sick infants who just need replacement 10 cc's per kilo every other day to DEHP. We can measure that and we can follow their reproductive and other outcomes and do lovely studies, because we are already following those kids anyway. And just sort of add that to the mix. I have read extensively and looked for

follow-up studies, and you find a little bit of human exposure data and no long-term follow-ups in babies or in hemodialysis patients or any of the multiply transfused patients. There is always the complication of sick people have lots of bad outcomes, but we are not even looking. So I think that this is a really wonderful opportunity to start looking.

DR. JACOBSON: I'd like to say that when we undertook the primate transfusion study and first evaluated all these transfusion studies so we would know the dosage to give the monkeys, we found that after 32 months at necropsy that we still had abnormal histology in these livers. Even though we did not see any other acute effects, we did still find this remaining. And we went and looked at some of our patients that were on hemodialysis, and we found that many of them had liver disease. It was not due to hepatitis B or non-A or non-B. We really don't know what it was due to. But you do see some of this and you begin to wonder is there DEHP implication in this.

DR. STRATMEYER: Dr. Luban?

DR. LUBAN: Yes. There are some patient groups that we have not mentioned that sort of goes to a little bit of 2B. So I will jump ahead if that is okay.

DR. STRATMEYER: Please.

DR. LUBAN: And it also addresses a little bit of what May stated, and that is sickle cell disease patients, who are often intermittently transfused and if they have had a stroke or had some secondary complications are chronically transfused. Also thalassemic patients who depend upon transfusion for their lives. These individuals often have significant liver disease and there has been hepatocellular carcinoma reported in heavily transfused thalassemic patients. However, I think one needs to be very careful when looking at that population as a study population because of iron overload and hydroxyl radical formation as well as potential concomitant viral hepatitis. But certainly the hepatitides that have been reported in that population, some of which have been explained and some of which have not been explained, it does provide an opportunity for a patient group that is probably very worth of study.

DR. STRATMEYER: Is one of the reasons why the lack of long-term epidemiologic studies because of the problem of confounding factors because so many of these people are -- again, as somebody said, these people are already sick? Is that one of the reasons why we see so few of these type of studies?

DR. LUBAN: Actually, I think it is that NIH

hasn't funded them.

DR. STRATMEYER: Do we have anybody from NIH here?

DR. LUBAN: Now there is about to be a thalassemia network that is going to be funded by NHLBI, and perhaps FDA needs to get together with NHLBI and see if one of the many outcome variables that is going to be studied in that population is not in fact plasticizer toxicity. It would provide a unique opportunity and it is already sort of a set aside fund. There are also sickle cell disease centers that are funded by NHLBI, and one of the many multi-institutional clinical studies that could be conceivably constructed is one in which one would evaluate, again, plasticizer toxicity in that population -- again, already partially funded. So you are not re-inventing the wheel.

There is a third population that we haven't mentioned at all, which is very critical and very vulnerable from a number of different perspectives, and more often than not transfused now, and that is fetuses who are undergoing transfusion for hemolytic disease of the newborn. There is no long-term outcome data on that population as a whole, and it is clearly vulnerable because you can't get much more premature than being a fetus. That is a population that perhaps also could be

studied.

DR. PHILLIPS: I just want to back up a little bit and address Dr. Orris's comments about shouldn't we address DEHP similarly to other pharmacologic agents that we use. I think certainly as a toxicologist, we consider all elements that are in someone. We don't necessarily label them drug/chemical substance, DEHP or whatever. A chemical is a chemical. It doesn't matter whether you sell it with a trade name or not.

DR. STRATMEYER: Excuse me. Could you get closer to the microphone? I think people are having trouble hearing you.

DR. PHILLIPS: Okay. Can you hear me now? I think the issue of potential chemical/chemical interaction, if you will, or drug/chemical interaction or substance/substance interaction is obviously an important point that Dr. Orris raised. I think, however, our clinical experience is that we haven't seen anything. So although I think it is an important point, after decades of use we should have seen something by now. I mean most important drug/drug interactions don't take decades to identify.

Speaking on point number 1, I think obviously a crucial point toxicologically is that it is

really the dose that determines the degree of toxicity. And very high dose studies in animals are not the same thing as clinically relevant doses. I think that is one aspect of how we can reconcile this issue. Other issues are inherent properties of the substance, the pharmacokinetics of oral versus parenteral administration, the route of administration likewise, and susceptibility of the host that is receiving the substance, whether it be animal or primate human or whatever you will. I think those are important points to look at. And I think that is perhaps why we haven't seen some of the effects in humans is dose and some of the other pharmacokinetic issues.

DR. SHEA: I just wanted to address your question about why this hasn't been looked at. I hate to burst your bubble, but not very many clinicians really -- maybe we were distracted by the HIV epidemic, but this has fallen off the radar screen. And just as a sort of natural experiment, I talked to a few of my colleagues at UNC and the head of nephrology doesn't worry about this, the head nurse in the adult dialysis unit doesn't worry about, the NICU attendings that I talked about don't worry about this. The only people I could find who worried about it were the IV pharmacists, and some of them do and some of them don't. So I think it is just an

issue of education and awareness. And part of that, I think, derives from the over-concentration on the carcinogenic endpoints and the failure to look at the other endpoints, which we are now beginning to appreciate.

DR. PHILLIPS: Yes, I'd have to agree with you. My appointment is in the department of pharmacology and toxicology, so in fact am the preacher most of the time about ADRs and drug interactions. And so you are right. Frequently it is overlooked but I think it is overlooked because it doesn't seem to be an issue. So people have probably moved on I suspect.

DR. ORRIS: If I might also -- I think you shouldn't be so harsh on yourself or our colleagues. If I might tell you a little story from my end of the world, environmental and occupational toxicology, if you will. By the way, a point on toxicology -- we only started our program of training toxicologists in the mid-1980's, by the way. So there is recent attention to this whole area. I would only tell you the story that several thousand years ago the Roman Empire fell. It was alleged that this was related to lead poisoning. Somewhat later, I went to medical school and in the early 1970's I was taught in medical school that we really didn't have to worry about lead poisoning in children and adults unless

you got to the 60 level or 70 level or unless they were seizing or in coma. In the time since I was in medical school until today, we now understand the toxicologic importance of lead, especially in the developing brain. We are now at a position where we don't quite understand how to identify a NOAEL level related to that, and we are concerned in children at levels at 5, 6, 10, 15 in this area. So I can only tell you that that is just one example. Asbestos is another. The fact that it hasn't become glaringly apparent to clinicians that work within a clinical paradigm of individual patients of the kind of subtle effects that we are looking at with these endocrine disrupters in the last 20 or 30 years should not give any of us cause for complacency. This is something we are beginning to understand and we are looking at now, and I think it is a wonderful opportunity to begin to make assessments that we have not been able to make before.

DR. SHEA: And we are just getting to the reproductive age of the kids who were exposed.

DR. STRATMEYER: Okay. Maybe we can move on to the third question. And this is one that should be interesting to try to answer, I guess. What is the significance of DEHP exposure from the therapeutic use of medical products such as blood bags when compared to

continuous or widespread environmental exposure. Anybody have the data?

DR. PHILLIPS: Well, I guess, Dr. Orris and I have to bite the bullet on this one and try to answer it. It is a very difficult question to answer. You are obviously not comparing apples with apples. One is either short-term high dose exposure to DEHP, for example from ECMO therapy, versus chronic low-dose exposure from everyday aspects of air, water and food. Though the environmental exposures from those media may in a lifetime add up to more milligrams, it is not necessarily the same thing as a total peak concentration, if you will. And it is very difficult to compare apples and applies. I think it is basically an unanswerable question from that standpoint.

DR. ORRIS: I find it hard to balance risk of one exposure versus another when we are dealing in one area. The same thing is said frequently about asbestos and mesothelioma frequently, and that is, gee, we are all exposed to it. It is so ubiquitous. We do find it in our sputum. We do find it around and in industrial cities as we walk around. That doesn't mean we don't want to make sure that there is not airborne asbestos in schools and in other locations in which populations are at particular risk. I think this is a similar kind of

discussion. That is, we don't want to add to the risk, if it is there, when there are alternatives or when we could put our mind to alternatives that do not contain that risk.

The other point, I think, that I don't want to lose from the day -- and I know it is not particularly relevant, but once again as someone concerned with the general environmental burden of these materials, it would be remiss for me not to mention that the reason at least some of us who are more generally concerned about the environment became concerned about the particular application and the phthalates involved in PVC plastic was from the original difficulty with respect to PVC plastic and its combustion and as a chlorine donor to the development of dioxins in general and that burden toxicologically on the globe today. And I think it is important that that issue -- that we don't lose that issue in the particular discussion as well on the phthalates.

DR. SHEA: I guess I would just add that the environmental exposures are estimated to be several orders of magnitude lower. They tend to be more oral, because food is the major environmental exposure. In children, they are disproportionately large according to some of the analyses compared to adults because diets are

different. Indoor air is also a source, which is very poorly studied, but the studies show that it can be sort of the second greatest exposure source. It is an inhalation. Again, it is probably disproportionately large in children because of their higher ventilation and their different activity levels. The medical exposures are IV, and that is very difficult to reconcile because we are infusing not only DEHP but the major metabolite, MEHP, every time we infuse blood. So we may bypass a lot of the transformation in the gut, but we are still giving the primary metabolite, which is generated in storage.

DR. PHILLIPS: I think one thing we have to keep in mind, and maybe as clinicians we sort of need to pat ourselves on the back up here a little bit, and that is in the big picture of healthcare, we know that life expectancy has never been longer than it is currently. We know that death rates have never been lower. We know that fertility really hasn't changed. Cancer death rates are not increasing. I think something we have been doing in the last -- certainly the life century when the life expectancy has almost doubled -- something we are doing seems right, something we are doing seems right. So if nothing else, I want to at least congratulate ourselves up here today. We seem to be doing something right.

DR. AUBUCHON: But all of us would like to

do it yet better.

DR. STRATMEYER: Let me move on. We have already gone into question 4 a little bit or touched on it. Let me get into question 4. Is there a need to look for adverse effects or the lack of adverse effects in humans that are chronically transfused with blood products prepared and stored in PVC DEHP bags? Here is a big, boy. How could such a study be designed and conducted in a way to provide meaningful data? Would significant data be generated by monitoring the health status of chronically transfused patients and comparing this to the health of the general population? Could meaningful data be obtained from normal, healthy donors of phoresis products, plasma or platelets who frequently donate up to two to three times a week for years and are exposed to small amounts of DEHP from the collection procedure? However, these individuals do not have underlying medical problems and their level of DEHP exposure from each procedure is small. What would be the appropriate clinical endpoint of these studies? Boy, there is a mouthful. I will turn it over to our experts here. We have people who have done a lot of clinical research, I think.

DR. AUBUCHON: I think Dr. Luban has already addressed some of these issues.

DR. STRATMEYER: Yes, she has.

DR. AUBUCHON: A federally-funded program is an absolute necessity, I think, to get anywhere. But even with a lot of federal money, this is still an enormous undertaking. Because individuals who are transfused, particularly individuals who are chronically transfused, are sick people and they do not have normal lifespans. They do not have normal functions in a number of different systems totally unrelated to the transfusions they have been receiving. This kind of study would require, in my opinion, comparison of a control group that was similarly exposed but possibly not to phthalate plasticizers, which would be very difficult to do currently in our current form of transfusion service delivery. So I am not quite ready to stand up and give you a five-minute oral presentation on the grant proposal I am about to submit on this. Because it would be about a five-hour presentation, I think, even if I did have it put together, which I don't. This is going to be an enormous study to try to tease out what is the effect of being transfused and chronically exposed to alloantigens and excess iron and a number of other factors in addition to being exposed to chemicals that are present in high concentration in blood bags.

DR. SHEA: We have a little bit of a model,

though, in Dr. Karle's study where she used the ventilator babies as the controls for the ECMO babies. And I think there are -- if were to employ sort of a multi-center trial approach, we could maybe do some very creative things and get maybe not perfect information, but a whole lot more than we have now. And I say again, since we are exposing these children, we can measure the exposures and follow outcomes. I think that it would be a lot of work and would require a multi-center approach, but I think that it is worth doing.

DR. STRATMEYER: The heparin-coated --

DR. SHEA: Well, she used -- well, the heparin-coated circuits were not used in vivo, I don't believe. She is still here, so she can correct me if I am wrong. But she compared -- she took very sick kids and the ones who were just a little bit sicker and couldn't be oxygenated using a ventilator, she put on ECMO and measured -- and used the almost-as-sick kids as controls. So they are still very sick and they still had all the other exposures. The one that you forgot is inter-lipid, which is a big one. Because it is run from a glass bottle, but it runs through PVC tubing, and in babies it goes through very slowly. It just sort of sits in the tube and drips in very slowly, and I would be stunned if DEHP wasn't getting in there.

DR. LUBAN: Well, I agree. I think a multi-institutional study, which might require about a year-and-a-half to put together could certainly be put together. I would also like to add that there is an ECMO registry of adverse outcome. However, it is not a registry that is utilizable for purposes of answering the questions that have been raised today, because it is acute outcome and not long-term adverse outcome. On the other hand, this is a relatively small group of investigators who do meet regularly and potentially could put together a longitudinal registry if people thought that that was a useful use of money.

DR. JACOBSON: Just a comment on the ECMO. I know that Dr. Karle's study and Dr. Schneider's study had different dosages. But the fact that Dr. Schneider at a very high dosage of DEHP calculated that -- saw jaundice in some of these infants, that maybe it is telling us something that there is some kind of dosage relationship here and that maybe we should be looking further by doing this type of study.

DR. ORRIS: If I could introduce a couple more complications to study design on this. What we know about some of these other endocrine disrupting chemicals, specifically some of the polychlorinated biphenols, is that the particular vulnerable period is fetal

development. The particular sensitive endpoint may well be diffuse neurotoxic effects manifested by behavioral changes or learning ability, et cetera. Having said that, I would only throw in that some of these populations may not be the best to study for those kinds of sensitive endpoints or for the period of exposure. I am not against any of the studies that have been pointed out, but I would certainly hope that someone would begin to explore the possibilities of IV usage during pregnancy and long-term review of the diffuse neurotoxic possibilities here.

DR. STRATMEYER: Okay. Let me move on to question 5, because I would like to open it up to the audience real soon. Again, we have already discussed around this question quite a bit, but if anybody has something else to add, let's do it. Has the current practice of transfusion medicine resulted in changes in the dose of DEHP that patients would receive from a transfusion of blood products?

DR. AUBUCHON: I can think of two things. One that I mentioned this morning, and that is the shift, which I think is pretty much universal in this country, away from packed cells to additive system red cells has resulted in a reduction dosage of infused DEHP by about 50 percent with retention or possibly potentiation of the

beneficial DEHP effect by concentrating the DEHP more in the red cells. So that would be the good news.

On the other hand, a potential downside of the practice that Dr. Luban mentioned a minute ago, which also seems to be quite universal, and that is dedicating one unit of blood to an individual neonatal recipient over the entire potentially 42 days of that unit for the time that that individual needs transfusion support might have resulted in increasing amounts of DEHP provided to that patient.

Because in the past, we would take a freshly collected unit of blood and split it amongst four or five satellite bags and transfuse all of those mini-units shortly or within a few days after collection before a lot of DEHP accumulation, and then the next day possibly expose the infant to yet another donor, but with relatively little DEHP. Now maintaining the unit for six weeks and giving it to one recipient repetitively through sterile connecting devices reduces donor exposures but may have actually increased DEHP exposure. True?

DR. LUBAN: Possibly. Not studied but possible. I guess the other issue is irradiation, which we have not touched base on. We do know that irradiation somehow or other alters red cell membrane, but that has been a very, very poorly studied area. We don't exactly

know how or why. And I don't know what effect irradiation might have on increased or decreased leaching.

DR. STRATMEYER: Okay. Anybody else?

DR. JACOBSON: I don't think there is any effect on the leaching with irradiation.

DR. SHEA: I was curious about irradiation too. Because we know heat increases it and we know agitation increases it and we know time increases it and we know lipid content increases it. I haven't seen anything about irradiation. Maybe the people in the industry can tell us if there have been some studies.

DR. STRATMEYER: Anybody in the audience?

DR. SHEA: I have to tell you just a funny story. When I finished my residency in 1981, we were still giving living donor transfusions to our babies. In other words, the pediatric residents would go get typed against a baby and then when a baby needed blood, we would draw 10 cc's out of our vein and push it right into the baby through a filter. So there was very little DEHP exposure, but there were a lot of other exposures that are no longer acceptable. So things have changed in 30 years.

DR. STRATMEYER: Okay. I guess we can open it up to the audience now. Do we have any questions for

the panel?

MR. BROWN: I know people are anxious to leave, but I will ask just two.

DR. STRATMEYER: State your name and --

MR. BROWN: Ron Brown, FDA. Thank you for reminding me. Actually a question for Dr. Shea and Dr. Luban. We had heard earlier Dr. Snyder tell us about decreased platelet aggregatability, at least in vitro, and that doesn't necessarily seem to carry over for in vivo studies. However, as I understand it, intercranial bleeding is one of the major complications for ECMO therapy, probably related to the heparinization of the patient primarily. But do you think the potential exists for a synergistic effect to occur between DEHP and heparin in potentiating intercranial bleeding in these patients?

DR. LUBAN: I have no idea. I mean, the incidence of intercranial hemorrhage in prematures is multifactorial. It certainly is exaggerated in ECMO infants, who usually are not premature, by the way, when they are put on because of the very high risk of intercranial hemorrhage. Certainly heparin is one part of it, platelet dysfunction is another, low coagulation factor secondary to decreased hepatic synthesis a third. There are any number of reasons as well as anatomical and

developmental reasons why babies have intercranial hemorrhage, and obviously it is something that we would like to avoid at all cost.

DR. SHEA: Just from this morning's presentation -- didn't Dr. Snyder and a number of other people point out that we get really nice bumps and bleeding stops with platelet transfusions even with the kind of loss of life and loss of aggregation or extra aggregation in vitro?

MR. BROWN: True. And I think I was just following on a theme that had been raised by other questioners in terms of a potential synergistic effect between drugs administered therapeutically and then incidental effect of the phthalates. The second question I had -- this morning in my presentation I had attempted to go through the thought process of the things that we were considering as we look at Dr. Jacobson's study from a regulatory perspective. I just wanted to ask Dr. Jacobson if she had anything to add or any advice that she could give the FDA in terms of interpretation of her results.

DR. JACOBSON: It's a wonderful study. We should do another one. I think overall the study in itself is very interesting because of the way it was done. I know the numbers are very small, but the fact

that we used plasma and platelets from donor monkeys that were collected and placed into bags with phthalate, you know regular DEHP bags. We had bags with polyethylene and those that were not transfused, and then looked at the liver parameters and used very sensitive tests. We used solubilized DEHP. It was done exactly the way we would transfuse children at our hospital.

And the fact that we did see the abnormal liver histology and some abnormal live BSP and clearance tests and technetium scans I think is telling us something is there. And the doses were very, very low and this persisted for 32 months after we stopped transfusions, we did see these abnormalities. I think that one should look at this and think about that maybe -- I realize it is not human, but it is as close as we could get. It was physiological and I think that we have to think about what is this study telling us. I think it is a relevant study. I think it is telling us that maybe we should think about alternatives.

DR. MIRIPOL: I might raise a question or two.

DR. STRATMEYER: Could you state your name?

DR. MIRIPOL: Jeff Miripol from Terumo.

DR. STRATMEYER: Thank you.

DR. MIRIPOL: Dr. Orris, I am a little

surprised, I guess -- maybe you are fairly new to this area. This is an area that has been discussed now for 25 or 27 years actually, and it strikes me that we have discussed over 25 years at various conferences issues about DEHP and its toxicity and its extraction and its excretion and its kinetics, et cetera. And, May, as you know, your study was done many years ago -- about 25 years ago.

DR. JACOBSON: That is right.

DR. MIRIPOL: And there was obviously critiques of it at that time and there certainly is some concerns about it now. I am a little concerned that we are not looking at some of these issues maybe with "modern" techniques or more recent advances, if you will, both in methods that are both analytical and experimental.

Again, it was a very small study and it troubles me that we kind of keep talking about it and other studies like it as well as studies that have used DEHP in inappropriate media. Again, I would critique some of the comments earlier when we are giving a lot of credence in terms of effects of toxicology from studies done in the early 1970's, when DEHP was given neat or DEHP was given in alcohol. I think they mean very little in terms of the real clinical effects. Why don't we

reproduce some of these studies in modern day fashion with appropriate methods. You know, we have been talking about this stuff for 25 years.

And frankly speaking from a manufacturing standpoint, we have looked at lots of different materials. Some of these new materials probably need to be studied in as extensive a form as DEHP has been studied and they have not been. So why don't we kind of like move forward and actually look at some of these studies in a more modern fashion and stop referring to frankly old studies which were probably done not in the proper fashion.

DR. STRATMEYER: Okay. Thank you.

DR. ORRIS: I am not clear if that was a response about the Roman Empire or not. Let me just say that coming from a broader public health perspective and looking at this more recently, I hope that we will -- those of us from a broader health perspective will be able to stimulate the interest that will secure some of the studies that you projected 25 years ago, as we have heard about today, to try to answer some of these questions that need to be answered. And I am very hopeful of that increased attention will move us in that direction.

DR. STRATMEYER: Any other questions? If

not, I'd like to thank all the panelists. I will now turn the show over to our host, Dr. Vostal.

CHAIRMAN VOSTAL: Well, I have the opportunity to provide a closing statement. First of all, I would like to say that we at FDA really appreciate the efforts that the speakers and the panel discussants have put forth to help us out with this difficult decision. We are going to be working on this continually, and I would like to tell you at least a little bit about where we stand and what our thinking is.

Well, I'd like to point out that the Center for Biologics is not going to be making any decisions in a vacuum. We have contacts with our sister agencies -- Center for Drugs, Center for Devices and Center for Foods. All these agencies have products that have plasticizer issues with them and we are in discussions with them and how they are dealing with their areas.

We also have interagency interest groups at NIEHS, the NICTR, Center for Toxicology, Center for Public Safety, and Center for Diseases. They also have plasticizer issues and toxicities and they are helping us in interpreting our problems. And together with the Center for Devices, I think that this workshop has been one of the initial steps for us to take to analyze what the current thinking is in terms of plasticizers and

blood component collection and storage.

Now the reason we have focused this workshop on blood collection sets and blood components is that the Center for Biologics and Office of Blood has regulatory responsibility for these products, and that is the blood collection sets, the tubing, the filter housings, blood component storage bags for red cells, platelets and plasma, and also for the anticoagulant bags. There is other issues that concern plasticizers, but we are trying to focus it, at least for our perspective, on blood storage and transfusion.

Now the CBER position on evaluating plasticizers. Of course, our primary concern is for blood product safety and efficacy. We believe that the current blood storage materials on the markets have been extensively studied and do have a long track record.

However, from what we are hearing here, there are issues that could be studied further. There may be problems that we have not looked at or not investigated, especially from the areas that we have heard about today in terms of reproductive toxicology.

The process that we are doing in terms of evaluating what is being used on the market is we are continuously collecting data on toxicity as it becomes available. And that is why we are very interested in

these risks assessments that are going on and in the new studies that are coming down the line that we will be able to look at and make further assessment in terms of human risk. We do encourage research into alternative plasticizers. There could be -- since there are potential underlying toxicity issues, this will be a way of decreasing or getting around those toxicity issues. And also as a benefit, maybe we will be able to find alternative plasticizers or products that can extend the efficacy of cell storage.

However, the down side of switching to a new plasticizer material is that we need to have these thoroughly investigated for both acute and chronic toxicity and also for their efficacy to store blood products. So as has been mentioned a number of times today, we don't want to switch from one well-studied material to one that is less studied which may have hidden toxicity we are not aware of.

In terms of when new products or new materials being available, we have certain guidelines that we have made available for the public to be able to follow our thinking in terms of being able to evaluate these products. The one that we are working on currently is the guideline on the content of non-clinical toxicology in clinical sections for applications

involving blood contact materials. This is being reviewed in-house and we hope that it will become available shortly. A guideline that is already out there is for platelets and it is for platelet testing and evaluation of platelet substitute products.

This was published this year and it is out for comment. It sort of tells -- it is a guideline to give an idea of what kind of platelet studies we would be looking at if a new storage system was introduced. And addition of at least this guideline and additional guidelines as they become available can be obtained at this CBER site.

So like I was saying -- let me also introduce to you the workshop planning committee. These people have been very involved for a long period of time and have provided tireless effort in getting this workshop on the way. You have met some of them already. They are Dr. Mondoro, Brenda Shafer, Betsy Poindexter, Sukza Hwangbo, and Joel Wilczek from the Center of Biologics.

From the Center of Devices, we have Dr. Mel Stratmeyer and Ron Brown, and we have been fortunate enough to be able to get help from Gary Moroff from the American Red Cross, who has given us a great historical perspective, both on plasticizers and in cellular blood

storage materials.

With that, I would just like to tell you that we appreciate all the discussion that was going on today. We are going to take a look at the transcripts and try to make a -- try to see which areas need further investigations and addressing. We look forward to working with you in the future on this issue.

Thank you very much.

(Whereupon, at 6:23 p.m., the workshop was concluded.)